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U.S. NATIONAL CANCER INSTITUTE

ANNUAL REPORT OF PROGRAM ACTIVITIES.

July 1, 1976 through Sept. 30, 1977

Part V

DIVISION OF CANCER RESEARCH RESOURCES & CENTERS

U.S. NATIONAL CANCER INSTITUTE

DIVISION OF CANCER RESEARCH RESOURCES AND CENTERS

ANNUAL REPORT

July 1, 1976, through September 30, 1977

TABLE OF CONTENTS

		Page
I.	Office of the Director	1
	Office of Program Planning	7
	Research Analysis and Evaluation Branch	9
II.	Biological Research Program	
	Cancer Biology Branch	
	Tumor Biology Program	13
	Immunology Program	27
	Cause and Prevention Branch	
	Carcinogenesis Program	37
	Cancer Epidemiology Program	51
	Viral Oncology Program	57
	National Organ Site Programs Branch	67
	National Bladder Cancer Project	69
	National Large Bowel Cancer Project	75
	National Pancreatic Cancer Project	81
	National Prostatic Cancer Project	85
III.	Centers and Treatment Program	
	Diagnosis and Treatment Branch	
	Clinical Oncology Program	91
	Cancer Diagnostic Research and Prevention Program	95
	Drug Development and Pharmacology Program	99
	Radiation Program	107
	Cancer Centers Branch Programs	111
	Research Facilities Construction Branch Program	115
IV.	Training and Education Program	
	Clinical Cancer Education Program	117
	Research Manpower Branch Programs	121

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267
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OFFICE OF THE DIRECTOR
DIVISION OF CANCER RESEARCH RESOURCES AND CENTERS

The Division of Cancer Research Resources and Centers is devoted to encouraging and supporting independently conceived and investigator-initiated laboratory and clinical cancer research. The Division is guided by the conviction that the most important new basic and clinical knowledge about cancer, as well as the most significant clinical applications of that knowledge, will be achieved in environments that encourage intellectual freedom and foster individual scientific initiatives. This conviction is fortified by the recognition that the vast knowledge gaps in both the biologic and clinical aspects of cancer are primarily fundamental. Therefore, advances against cancer require involvement and interaction of many independent minds and implementation of the best research ideas that can be brought to bear on the basic biology and fundamental clinical problems. Accordingly, the Division of Cancer Research Resources and Centers fosters the competitive application of the Nation's outstanding scientific initiatives and technical capabilities in the effort against cancer. This approach is most evident in DCRRC's Traditional Research Programs, but the same considerations provide the principal guidance for all of the Division's activities.

Traditional Research Programs

The Traditional Research Programs of DCRRC support investigator-initiated and investigator-conceived research by awarding regular research project grants and program project research grants. These programs encompass carcinogenesis, drug development and pharmacology, epidemiology, tumor biology, viral oncology, immunology, diagnosis and detection, clinical oncology, radiation therapy, and radiation biology and physics. These grant programs avail themselves of the broadest possible scope of research ideas and technologies by accepting for consideration unsolicited grant applications in very broadly defined areas of cancer relevance. The most significant and most feasible research plans are selected for support through the peer review system.

Organ Site Research

The National Organ Site Cancer Program extends the reliance on investigator initiatives to research program planning and administration in National Organ Site Cancer Projects. There are National Organ Site Cancer Projects for

Bladder, Large Bowel, Prostatic, and Pancreatic Cancer. In each case, NCI has delegated many of the administrative and scientific responsibilities to a non-NCI National Project Director who is advised by a working cadre of scientists from many research institutions. National Projects are planned and targeted, but they rely on investigator initiatives to fulfill their targeted research programs.

Manpower Programs

The Division assures the development of cancer research talents and clinical cancer expertise in its Research Manpower Programs and its Clinical Cancer Education Program. These endeavors encourage the initiatives of the Nation's scientists, teachers, and educators by accepting unsolicited applications, and by identifying innovative cancer research training and cancer education through peer review. The Research Manpower Programs provide support for training in the many laboratory and clinical research disciplines relevant to cancer cause and prevention, cancer detection and diagnosis, and cancer therapy and restorative care. Research training initiatives are supported by Institutional and Individual Fellowship awards as well as by Research Career Development Awards. The Clinical Cancer Education Program provides grants to support improved cancer teaching primarily in institutions which provide education for physicians and dentists. The Program's goal is to provide future health professionals properly educated to provide superior care and treatment to cancer patients.

Construction and Cancer Centers

Both the Construction Program and the Cancer Centers Program are devoted to establishing research environments in this country that promote innovative clinical and laboratory cancer investigations. Although there are NCI guidelines for exploratory and cancer support (core) grants, and there are NCAB criteria for comprehensive cancer centers, the program's primary concern is the development of milieus appropriate for independent initiatives in cancer research and education.

The Cancer Centers Program continues its endeavor to formalize the evaluation of prospective comprehensive cancer centers. These evaluations which incorporate site visits and involve the National Cancer Advisory Board have been extended to assessing the status of previously recognized comprehensive cancer centers. The assessments, when completed, will provide NCI and the National Cancer Advisory Board a detailed understanding of NCI's overall Comprehensive Cancer Centers Program. They will also provide NCI with objective opinions regarding strengths and weaknesses to share with the Directors of comprehensive cancer centers.

On December 30, 1976, the University of California, Los Angeles, was recognized as a comprehensive cancer center. There are now 19 recognized Comprehensive Cancer Centers.

COMPREHENSIVE CANCER CENTERS

Colorado Regional Cancer Center, Inc.	Denver, Colorado
Comprehensive Cancer Center of Florida	Miami, Florida
Duke University Comprehensive Cancer Center	Durham, North Carolina
Fred Hutchinson Cancer Research Center	Seattle, Washington
Georgetown University/Howard University Comprehensive Cancer Center	Washington, DC
Illinois Cancer Council	Chicago, Illinois
Johns Hopkins Oncology Center	Baltimore, Maryland
Mayo Comprehensive Cancer Center	Rochester, Minnesota
Memorial Sloan-Kettering Cancer Center	New York, New York
Roswell Park Memorial Institute	Buffalo, New York
Sidney Farber Comprehensive Cancer Center	Boston, Massachusetts
The Fox Chase/University of Pennsylvania Comprehensive Cancer Center	Philadelphia, Pennsylvania
The Ohio State University Comprehensive Cancer Center	Columbus, Ohio
The University of Texas System Cancer Center	Houston, Texas
University of Alabama Comprehensive Cancer Center	Birmingham, Alabama
University of California, Los Angeles Comprehensive Cancer Center	Los Angeles, California
University of Southern California/ Los Angeles County Cancer Center	Los Angeles, California
University of Wisconsin Clinical Cancer Center	Madison, Wisconsin
Yale University Comprehensive Cancer Center	New Haven, Connecticut

Young Investigators Awards

The Division developed guidelines and announced plans to establish the Young Investigators Awards Program. These awards will provide a maximum of $25,000 a year for up to three years for young investigators to initiate independent research. The Institute plans to make approximately 25 such awards each year.

Cancer Research Emphasis Grants

The Division continues to coordinate NCI's Cancer Research Emphasis Grants (CREG). DCRRC provides guidance in policy and procedures for the NCI Divisions that use CREG. Cancer Research Emphasis Grants are solicited by Requests for Applications (RFA's) which designate the research desired but leave approaches and methodologies to the scientists who apply. The following table summarizes CREG awards in fiscal year 1977.

CANCER RESEARCH EMPHASIS GRANTS

TRANSITION QUARTER AND FISCAL YEAR 1977
(Dollars in Thousands)

NCI DIVISION	TRANSITION QUARTER		FY 1977	
	No.	Amount	No.	Amount
DCBD	0	$0	6	$450
DCCP	12	1,056	72	5,700
DCT	9	1,196	17	1,674
TOTALS	21	$2,252	95	$7,824

Budget

Funds available to support the programs of DCRRC in the transitional quarter of fiscal year 1976 amounted to $67,299,000 and the funds available during fiscal year 1977 amounted to $342,474,000. The distribution of those funds was as follows:

BUDGET DISTRIBUTION

TRANSITION QUARTER AND FISCAL YEAR 1977
(Dollars in Thousands)

PROGRAM	TRANSITION QUARTER		FY 1977	
TRADITIONAL	$22,449		$132,538	
SCIENTIFIC EVALUATION	412		1,500	
CONFERENCE GRANTS	0		1,246	
TOTAL REGULAR PROGRAM		$22,861		$135,284
CORE	12,684		56,700	
EXPLORATORY	279		1,200	
CCPDS	0		1,431	
TOTAL CENTERS SUPPORT		12,963		59,331
PROGRAM PROJECTS		22,629		80,653
RADIATION DEVELOPMENT		256		4,175
RCA	28		240	
RCDA	146		3,304	
TOTAL CAREER PROGRAM		174		3,544
BLADDER	614		4,038	
LARGE BOWEL	409		5,293	
PROSTATE	589		3,435	
PANCREAS	104		1,934	
TOTAL ORGAN SITE		1,716		14,700
CLINICAL EDUCATION		7		8,996
FELLOWSHIPS		3,193		18,236
GRADUATE TRAINING	0		788	
CLINICAL TRAINING	0		767	
TOTAL TRAINING		0		1,555
CONSTRUCTION		3,500		16,000
TOTAL DCRRC		$67,299		$342,474

With the funds available in fiscal year 1977, the Division made 2,867 awards and 412 supplements. The following table shows how the awards were distributed among noncompeting projects, competing renewals, new projects, and supplements. The table also compares the amounts requested with the amounts approved and amounts awarded.

FISCAL YEAR 1977

DISTRIBUTION OF AWARDS
(Dollars in Thousands)

	REQUESTED		RECOMMENDED		AWARDED		UNFUNDED	
	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT
TYPE 5 (non-competing)	2,059	$233,724	2,059	$233,724	2,059	$233,724	0	$0
TYPE 2 (competing renewals)	777	148,494	598	76,411	256	53,552	342	22,859
TYPE 1 (new)	2,648	290,564	1,637	117,254	552	46,645	1,085	70,609
TYPE 3 (supplements)	(105)	9,550	(455)	12,095	(412)	8,553	(43)	3,542
TOTALS	5,484	$682,332	4,294	$439,484	2,867	$342,474	1,427	$97,010

Staff

During the year, 12 people left the staff and 27 new employees joined the staff of DCRRC. At the close of the fiscal year, DCRRC's total staff numbered 171.

OFFICE OF PROGRAM PLANNING

The Office of Program Planning is located in the Office of the Director, DCRRC, and is responsible for the planning and evaluation of grant-supported programs administered by the Division.

The Office of Program Planning (OPP) integrates data from various sources within the Division in order to establish a broad overview of past trends and present and future needs. It also serves the National Cancer Institute (NCI) as a whole by maintaining a close working relationship with the Office of Program Planning and Analysis (OPPA) in the Office of the Director, NCI.

To help fulfill many needs of the Division, OPP administers and manages contracts with outside firms and cooperates with the OPPA in several other contract-supported studies. Some tasks accomplished with the aid of these contracts follow.

HSA Review

The OPP led a study to review and evaluate the impact of the National Health Planning and Resources Development Act of 1974 (P.L. 93-641) on the NCI and the NIH. This Act created new regulatory requirements which have implications for both NIH and its grantees and contractors. The legislation gives health systems agencies (HSA's) across the country review functions which may impact on NIH projects involving:

- development, expansion, or support of health resources (i.e., services, manpower, and facilities);
- capital expenditures related to development of or changes in health-care facilities and services;
- increase, conversion, or relocation of beds; and
- new health services.

Because P.L. 93-641 is being implemented in phases, its potential impact on NIH is developing incrementally as different sections of the law come into effect. Continuing efforts are necessary, therefore, to assist NIH staff and constituents in understanding the legislation's impact at each stage of implementation, and to determine what actions, if any, must be taken to comply with the law.

During fiscal year 1977 an analysis of the health planning legislation and strategies for dealing with the requirements of P.L. 93-641 was prepared. NIH program directors were interviewed to determine which extramural activities would be most affected by HSA review. The analysis includes an examination of the NIH grants and contracting processes to develop recommendations for a system to screen incoming applications and proposals for compliance with HSA regulatory requirements. Possibly the most critical task is the

preparation of a document which will explain the unique needs and concerns of research investigators and institutions to the health planning agencies. This document will serve as the basis for any future local or state review of NIH grant applications and proposals.

Patient Management Study

An ongoing study attempts to develop a methodology for documenting current patient management practices in institutions treating cancer patients. It is expected that the methodology will aid in measuring the rate at which new research knowledge is translated into cancer patient care. The methodology is designed to utilize data available in hospital medical records that can be abstracted by trained nonphysician personnel.

Other Studies

The OPP continues to work closely with DCRRC scientific and management staff in assessing program area needs. During the past year, specific program tasks involved the following areas: immunology - conference evaluation; clinical oncology - assessment and management system; radiation oncology - equipment needs and development of proposed regulations; centers and construction - continuing long-range planning; clinical cancer education and research manpower - amendment to ongoing clinical oncology needs study and proposed research manpower needs; and research analysis and evaluation - methods for evaluating technology transfer.

RESEARCH ANALYSIS AND EVALUATION BRANCH

History and Content of Program

The Research Analysis and Evaluation Branch analyzes and indexes the scientific content of all programs supported by the DCRRC, all NCI contracts, and all NCI intramural projects. The Branch serves as a major source of scientific information concerning NCI-supported research projects, and as a point for disseminating that information to other Divisions of NCI, the Office of Cancer Communications, the NCI Financial Management Branch, the NCI Program Analysis and Formulation Branch, the International Cancer Research Data Bank, and other NIH organizations such as the Research Documentation Section and the Research Analysis and Evaluation Branch of the Division of Research Grants (DRG), and other NIH Institutes.

Pending grants and contracts are compared with existing NCI-supported projects at the same institution to assure that there is no overlapping or duplicated support for a research project.

Research proposed in applications that are awarded is analyzed by the Branch's scientific staff, indexed, and stored in computer-searchable form. The Smithsonian Science Information Exchange (SSIE) abstracts supplied by the applicants are also stored in this system. The grant administrative data from DRG's IMPAC system are also stored in the Branch's system and can thus be linked to the scientific data in various combinations of desired information.

Published results from cancer research conducted under the grants of DCRRC are monitored through the Branch literature surveillance program. Approximately 300 major medical and scientific journals are scanned to identify papers resulting from NCI grant-supported research. These references, and additional references obtained from reprints submitted by grantees, are arranged by DCRRC program areas and the list is issued monthly with a subject index as the NCI Grant Supported Literature Index. The Index is the most complete record of accomplishments of the total extramural research programs. In calendar year 1976 approximately 4,900 articles, representing the work of 8,596 authors appeared in the Index. Retrospective searches can be performed using a tape version of the Index.

Narrative reports on selected areas of cancer research are prepared by the Branch at the request of DCRRC staff or on the initiative of Branch scientific staff members.

Accomplishments

The Branch has continued to enter scientific indexing for funded NCI grants into its automated system begun in 1975 to replace the edge-punch card system previously used. Approximately four-fifths of the active grants have been entered into this system. The system, which is used to respond to inquiries and to prepare reports, includes three types of data: administrative (from

DRG's IMPAC System), scientific indexing, and SSIE abstracts. However, the Branch only supplies the scientific indexing directly. Each grant is indexed according to a list of fixed terms compiled by the Branch; in addition, as many as 60 other, nonfixed key words and/or phrases can be used to index each grant, thus providing a specific and detailed subject analysis of the project.

The system, Grants Elements Network-Internal Users System (GENIUS), is composed of three separate files: the active grants file, the inactive grants or history file, and the unfunded grants file. All scientific and administrative data on both the active and the inactive files, except for the SSIE abstracts, are searchable. The unfunded grants file consists of disapproved and approved-but-not-funded grants for the past two years. This file contains only administrative data from IMPAC plus birth dates of the principal investigators, with project title, program area, and study section as the only points of access. GENIUS is maintained and operated solely by the Branch, which is greatly indebted to the Division of Computer Resources and Technology, NIH for help in developing and implementing the system.

Many subject analyses of NCI-supported research were prepared by the Branch during the past year; these included topics such as nuclear medicine, hormone receptors, interferon, CAT (tomography), radiosensitization, and the relationship of cancer epidemiology to nutrition.

One narrative report was prepared during the past year:

Photodynamic Effects of Light-Activated Dyes:
Potential for Cancer Therapy and Related Research.

Projections

The Grant Elements Network-Internal Users System will be expanded to include a fourth file--Intramural Research Projects. This file contains the same kind of data as the active and inactive grants files. The scientific indexing of Intramural Research Projects is approximately one-half completed, and retrieval programs to access this material are being tested. An interim file will be set up for active contracts until scientific indexing has been completed for active research grants. When the grants have been indexed, comprehensive scientific indexing of the contracts will ensue, using the format now used for grants (fixed terms plus key words and/or phrases). The final file to be added to GENIUS will contain training programs information.

Other Activities

The Branch maintains regular liaison with other Divisions of NCI. Extra copies of grantees' reprints are sent to each Division, as are copies of the SSIE abstracts for all grants. SSIE abstracts of grants concerned with specific research areas are sent on a regular basis to NCI staff who have requested this service.

Staff members also serve on Divisional and Institute Committees (e.g., Steering Committee of the Breast Cancer Task Force and OCC Ad Hoc Group on Cancer Information Clearinghouse Operations) which attempt to foster better communication concerning NCI-sponsored research and to promote research in areas judged especially significant to the National Cancer Program.

TUMOR BIOLOGY PROGRAM

Description

The Tumor Biology Program was created in 1974 to encompass and develop basic biological and biochemical research in an area of cancer biology specifically devoted to the study of tumor cells and transformed cells. Consequently, there is very little emphasis in the program on studying the process of tumor development, and there is considerable emphasis on identifying unique properties of cancer cells that can be translated to clinically oriented programs and then tested for their potential in cancer treatment, therapy, and diagnosis. The major long-term objective of the program is to develop a basic understanding of the biological processes in malignant cells that account for loss of growth control and lead to penetration and invasion of surrounding tissues. Approximately ninety percent of the research supported is directly concerned with comparative studies of normal and cancer cells. Ten percent is focused on biological systems and technological developments believed to be highly relevant to or required for the eventual success of the other research areas in tumor biology.

In the last few years it has become increasingly apparent that nearly all of the processes of cells that contribute to cell division, cell movement, and cell recognition are highly integrated and interdependent. Because tumor cells are altered throughout, it is important to discern how the complexity of cell surface-cell cytoplasm-cell nucleus integration is disrupted and changed to produce a cell which no longer conforms to normal controls. Thus, tumors, once established, are diseases which will very likely require a complete understanding of total cell functions if more successful therapeutic and diagnostic measures are to be developed.

Although an integrated concept of cell function is required to understand tumor abnormalities, the Tumor Biology Program is categorized in several major research areas. The area currently receiving the greatest emphasis is membrane biology. This emphasis has been established for two reasons. First, alterations of the cell surface membrane appear to affect the ability of a cell to divide; the specificity and strength of cells' interactions with other cells; the ability of a cell to move through intercellular spaces; and cells' capacities to ingest nutrients and respond to factors in the environment. All of these cellular properties are altered in a malignant cell. Second, greater knowledge of cellular surface properties may help identify determinants that can stimulate the immune system and thereby stimulate a natural defense against cancer. The regulation of gene expression is important to understand because there is every indication that genes not expressed in normal cells become active in tumor cells. Understanding gene regulation may suggest specific sites in cells that can be treated to control tumor progression. Nutritional factors and growth factors present in the blood may be altered through diet and hormonal manipulation to stem tumor growth. Therefore, knowing the growth requirements of tumor cells is essential. Research in cancer genetics is developing rapidly. Improvements in technology and experimental approaches may help reveal specific chromosome abnormalities which

indicate a predisposition to cancer. Somatic cell genetics, somatic cell hybridization, and DNA recombination will undoubtedly become powerful tools for learning more about gene regulation and for identifying the number and specific chromosome changes responsible for neoplasia. Knowledge of tumor cell products is increasingly important because some of these products increase the chances of tumor progression while others can be early indicators of the presence of tumors. Understanding cancer cell metabolism may reveal important enzyme reactions in the tumor cell that can be specifically inhibited to stop tumor growth and/or help in the design of chemotherapeutic agents.

In all of the above areas model systems are being developed and studied that enhance the tumor biology research effort. Some of the more significant examples are hepatomas that exhibit 20-fold differences in growth rate, melanomas that exhibit different degrees of metastatic potential and organ site preference, neuroblastomas that show varied differentiation potentials and tumorigenicity, teratocarcinomas that are reversible from the differentiated state and the cancerous state, and virally transformed cells that mimic many of the growth properties and social interactions of tumors.

Accomplishments

Membrane Biology: Membrane biology problems are difficult to approach experimentally and conceptually in interpreting malignant cell behavior. The past year has not produced major advances in our understanding of cancer cell membranes, but it has produced numerous attempts to consolidate and interpret existing information. It is increasingly apparent that the cell surface is under strict and coordinated cytoplasmic control. Complicated events that result in unequal distribution of specific surface proteins, cell adhesion, and cell locomotion require interactions of cell surface components with cytoplasmic microtubules (a skeletal framework which contributes to cell rigidity and structure) and microfilaments (muscle-like contractile fibers without which a cell cannot move effectively). Alterations in the cell skeleton, the cell contractile elements, and the distribution of cell surface proteins accompany neoplastic transformation. Perhaps, a breakdown in communication between the cell surface and the cytoskeletal-contractile apparatus is the most important change contributing to the cancer cell's loss of growth regulation, loss of cell-cell recognition controls, acquisition of invasive properties, and escape from the host immune system (Nicolson, 1976a; Nicolson, 1976b). The observation that a single viral gene product can alter many membrane properties, is consistent not only with a single common cause producing many different effects but also with a general breakdown of cell surface proteins by proteolysis or disruption of normally functioning protein complexes (Hynes, 1976; Hynes and Pearlstein, 1976; Pearlstein et al., 1976). In noncontacted states and during cell division, normal cells share many common characteristics with malignant cells. When a normal cell contacts another cell, the uptake of nutrients declines, cell proliferation becomes controlled, and the concentration of key metabolic secondary messenger molecules is elevated. However, malignant cells fail to develop mechanisms for controlling membrane organization and function and are unable to avoid continued growth upon contact. Several known membrane-associated components may require organization for normal function and could account for many of these

observations (Scott and Furcht, 1976). The current consolidation of information and conceptual model building will undoubtedly encourage more critical and revealing experiments and improved technology.

Considerable evidence indicates that changes in the carbohydrate portion of surface membrane glycoproteins are responsible for the malignant cell's loss of contact inhibition of growth and inability to interact specifically with other cells in the environment. Significant progress in this area is anticipated. One experimental problem is to identify the changes that take place, and also to artificially create protein changes and observe cell behavior. An inhibitor of protein synthesis has been tested which prevents the resynthesis of glycoproteins that lack specific parts of the carbohydrate portion (Brinkerhoff and Lubin, 1977). Controlled inhibition of glycoprotein synthesis may represent another important tool for examining their biological importance. Changes in the patterns of mannose-containing glycoproteins are growth dependent (Muramatsu et al., 1976). These surface glycoproteins may have important implications in growth regulation and in the immunogenicity of cells. Changes in membrane glycolipids also occur during tumorigenesis. A specific glycolipid has been isolated and characterized as a novel component of human colon cancers that are highly metastatic. This glycolipid does not accumulate significantly in nonmetastasizing tumors (Watanabe et al., 1976). Also, the pattern of long chain glycolipids is predictably altered in highly tumorigenic L cell lines as well as in their low and high tumorigenic hybrids (Itaya, 1976). If these observations represent general characteristics of highly malignant cells, they could be important in diagnosis and in planning on proper therapy.

Somatic mutants of Chinese hamster ovary cells can be obtained which are defective in their attachment to collagen, a basic surface constituent of contact inhibited cells (Klebe et al., 1977). Because transformed cells are much less adhesive to each other and to other cells than are normal cells, these defective cell lines can be studied in regard to the role of specific glycoproteins in the adhesion process and whether an increase in collagenase activity, a proteolytic enzyme, can account for loss of adhesion. If collagenase is involved, it may be possible to develop specific inhibitors for this enzyme and possibly control or reverse tumorigenesis.

Regulation of Gene Expression: Whether a cell is normal or neoplastic, only a small proportion of its genes are being expressed at a given time. How the regulation of gene expression is achieved in mammalian cells remains an important question for cancer research. Many different processes can control gene expression, and it appears that all of these processes are at work simultaneously. Gene expression can be controlled at the level of transcription (i.e., synthesis of RNA), RNA processing, RNA translocation from the cell nucleus to the cell cytoplasm, or at the level of translation. More than 150 cellular components are involved in the expression of one gene, and alterations in any one of these components could be a major factor in neoplasia. Unfortunately our knowledge of what gene(s) and gene product(s) contribute to tumorigenesis and how the regulation of mammalian gene expression becomes aberrant remains incomplete.

During growth, the cells pass through several different steps distinguished by quiescence, gene expression, and gene replication. All of these steps are under strict control even in a neoplastic cell which divides continuously. Different steps in the cell cycle are regulated by gene products on different chromosomes (Ming et al., 1976). Before proliferation begins, gene activity increases, and this takes place mostly in the nucleolus (Baserga et al., 1976). This sustained gene activity, and subsequent gene duplication, require the accumulation of nuclear proteins, probably non-histone chromosomal proteins (Whelly and Baserga, 1977), yet, a few specific non-histone proteins are lost from chromosomes in rapidly growing cells (Yeoman, 1976). Non-histone proteins are the best candidates for specific gene regulation, but confirmation of their regulatory role awaits further progress on the histones purification, a better understanding of their modified forms (e.g. acetylation, phosphorylation), and controlled experimental systems for assaying their biological activity.

Differences in gene expression result from altered RNA polymerase activity during transcription. When the polymerases of a poorly differentiated liver tumor are compared to those of normal liver, significant differences are observed (Rose et al., 1976). However, it remains uncertain whether these alterations in RNA polymerases are specific to malignant cells or are simply related to growth in general.

Nuclear Poly (A) polymerases have also been compared in the hepatoma model system and differ structurally and in their sensitivities to acid and base (Rose and Jacob, 1976). If these changes result in more efficient or more selective processing of the messenger RNA in hepatomas, they account for the drastic changes in gene expression that characterize cancer cells.

A new DNA polymerase is expressed in a series of hepatoma cells and it does not appear in the normal adult liver. This enzyme activity was also found in embryonic tissue (Chiu and Hnilica, 1976). If the neoplastic enzyme responds to specific inhibitors that normal proliferating cells are resistant to, it could represent a potential site for chemotherapy.

In addition to histones and non-histones, there is a class of small nuclear RNA molecules that could be important in controlling gene expression. Two conflicting observations must be resolved. The pattern of the small RNA molecules' associations with mitotic chromosomes suggests to one investigator that they may specify which genes are expressed (Goldstein, 1976). Another investigator clearly demonstrated that small RNA molecules are not restricted to the nucleus but are also associated with other subcellular structures. Some small nuclear RNA's may have a structural role in the nuclear skeleton rather than in regulating gene expression (Zieve and Penman, 1976).

Another important approach to understanding gene regulation is to examine changes that occur in the synthesis of a specific gene product as a result of malignant transformation. Clearly the lack of tryptophan oxygenase in hepatoma cells does not result from impaired hormonal regulation but does result from nonmessenger RNA synthesis (Murthy et al., 1976). Therefore, differences observed in Poly (A) polymerases may be irrelevant if this result holds for

all gene products that are not expressed in neoplastic cells. Current studies of the regulation of specific gene products such as Bα-fetoprotein, α-globulin, and albumin should answer this question.

A number of model systems are being developed and used to determine factors that contribute to regulating specific gene product synthesis (e.g. Friend Leukemia Cells, neuroblastomas, teratocarcinomas). Considerable progress is expected using neoplastic cells that also exhibit many differentiated functions. The teratocarcinoma is interconvertible between the neoplastic and several differentiated states, and scientists are learning how to control this development.

Nutritional Factors and Growth Factors: The possibility of using natural factors to influence the growth rate of cells for the control of cancer is receiving increased attention. The factors could be nutritional, they could be hormones, or they could be ubiquitous protein components of serum (Lipkin et al., 1976). Mammalian cells grown in culture, which can produce tumors when injected into a host animal, are excellent model systems for examining the effects of natural factors on cancerous growth. Currently nearly all basic biological research is concentrated on determining what factors in culture are required for maintaining, stimulating, and inhibiting cell growth (e.g. Brooks, 1976). Some of these factors are in serum and some are actually produced by the tumor cell. When these factors are identified and when their modes of action are understood, the next step will be to biochemically control their concentrations in serum and determine which ones are essential for maintaining tumor growth and which ones can inhibit cell division. Much is known about the growth requirements of cultured cells grown under artificial conditions, but there is a great need to extend this knowledge to animal model systems.

The potential of using nutrition to control tumor growth is under investigation. Mammalian cells can use 32 different carbohydrates as energy sources. A study has determined the carbohydrate preferences of several different mammalian cell lines, some of which are malignant. Only fifteen carbohydrates supported cell proliferation, and forty-two others were toxic or inhibited growth. Furthermore, the ability to utilize certain carbohydrates distinguished some cell lines from others (Burns et al., 1976). Cultured cells, when exposed to different carbohydrates, exhibit significant changes in their membrane structure (Amos et al., 1976). Unsaturated fatty acids, when supplied to cultured cells, stimulate growth 5-10 fold (Messmer and Young, 1976). Both fatty acids and carbohydrates are essential constituents of membrane structure and they can be easily manipulated in the diet. Research is needed on specific nutritional manipulations that can limit the growth and survival of malignant cells without injury to normal cells.

Normal cells in culture stop growing once they make contact while malignant cells do not. A factor isolated from the culture medium of normal hamster melanocytes can restore contact inhibition to malignant hamster melanocytes. This hamster factor also is effective on malignant melanocytes of human and murine origin (Lipkin and Knecht, 1976). Melanocyte contact inhibition factor (MCIF) is the first mammalian cell glycoprotein identified with this

kind of activity. It may be the prototype for a critical class of closely related, if not identical, surface-active molecules concerned with regulation of normal cell-cell interactions.

A number of animal serum factors have growth-inhibitory or cytotoxic effects on tumor cells in culture. A serum factor isolated from normal patients inhibited the growth of lymphocytes from patients with chronic lymphocytic leukemia but had no effect on those from healthy donors. (Monahan et al., 1976). These results suggest factors in normal individuals monitor neoplastic growth without affecting normal growth. If this is true, understanding how the levels of these factors are regulated in the serum is an important task for cancer research.

It remains important to purify, characterize, and to discover how tumor angiogenesis factor (TAF) acts. Unfortunately, progress has been slow because its purification is difficult. This factor promotes the vascularization of solid tumor tissue which provides a better supply of nutrients and increases the possibility of further growth and transition to malignancy. Recently an endothelial cell culture system has been defined for investigating the detailed mechanism of TAF action (Fenselau and Mello, 1976). In addition, attempts have been made to suppress the vascular response initiated by tumors with glucocorticoids. Six-S-methylprednisolone decreased and sometimes totally inhibited the vascularization phenomenon using the hamster cheek pouch assay (Shubik et al., 1976).

Cancer Cell Genetics: As the genetics of mammalian cells becomes amenable to experimental approaches, cancer biology will progress more rapidly. The combined use of genetics and molecular biology for elucidating biological processes in simple organisms such as bacteria is the most successful approach ever used to advance our understanding of living organisms. Although considerably more complex, experimental mammalian genetics will surely contribute as much to our understanding of biological processes in mammalian cells. Mammalian cells that require certain amino acids for growth are becoming available (e.g. Naylor et al., 1976; Hankuison, 1976). It is possible to produce somatic cell hybrids in culture between mouse cells and malignant human cells. The advantage of using hybrids between different species is that often only one human chromosome is retained (e.g. Knowles et al., 1977). There is little doubt that the transition from normal to malignant cell involves alterations of cellular regulation events stemming from permanent changes in the cell genome. Continued improvement in somatic cell genetic and somatic cell hybridization methodology can reveal which chromosomes and genes contribute to tumorigenesis, as well as aid the molecular biologist in defining the specific gene products that upset normal cell regulatory processes.

Somatic cell genetic selection has provided an entire set of melanoma variants that not only exhibit widely different degrees of metastasis but also high degrees of organ selectivity for metastasis (e.g. predominantly in lung tissue) (Nicolson et al., 1976). The isolation and characterization of these variant cell lines with enhanced metastatic properties offer an excellent model system for studying surface characteristics of the malignant neoplastic state.

Normal human cells have been hybridized with highly malignant HeLa variant cells. Malignancy, as measured by tumor formation in appropriate host animals, is suppressed in malignant-normal hybrids. However, malignant-malignant hybrids always retain their malignant character. These studies clearly demonstrate that malignancy behaves as a recessive trait in human cells (Standbridge, 1976). If nonmalignant hybrids derived from cancer cells retain the antigenic identity of the malignant parent, they may be useful in cancer immunotherapy.

Classical genetic studies show that patients who exhibit certain autosomal recessive syndromes are predisposed to cancer (Swift, 1976). Progress in identifying genetic predisposition to cancer and leukemia depends on clarifying the genetics and epidemiology of recessive syndromes associated with malignancy and on establishing new associations through continued investigations.

Cancer Cell Metabolism: Cell metabolism in the Tumor Biology Program is concentrated primarily on enzymology as it is related to interconversions and syntheses of small molecules (e.g. sugars, fatty acids, nucleotides) and degradation and turnover of larger molecules (e.g. proteins, nucleic acids). Many of the current cancer chemotherapies depend on effects of drugs on critical enzymes in synthetic metabolic pathways. The basic premise is that certain enzymes in cancer cells can be selectively inhibited without affecting normal cells. To date, no chemotherapeutic agents completely exhibit this selectivity, and damage to normally growing cells remains a substantial problem for continued research.

Aspartate transcarbanylase is one of the key regulatory enzymes in the pyridine nucleotide biosynthetic pathway. Theoretically, if cancer cells are prevented from making pyridine nucleotides, they will cease to grow. Recently, it has been shown that N-phosphonacetyl-L-aspartate (PALA) inhibits aspartate transcarbanylase and exhibits antitumor activity (Johnson et al., 1976). PALA is being evaluated as an antitumor agent. In another study mutant mammalian cells are developed which overproduce and underproduce aspartate transcarbanylase. These cells will be used to learn how to control the enzyme synthesis rather than trying to inhibit its enzymatic activity. Stable over-producing mutants have been obtained (Kempe et al., 1976). This is an example of how problems of metabolism can be dissected using somatic cell genetics. It also demonstrates how the biological problems of cancer are interrelated since the latter approach is a problem of regulating gene expression.

It has long been known that energy metabolism in malignant physiology favors glycolysis rather than aerobic respiration. Mitochondria, which are responsible for respiratory metabolism, have been studied in normal liver and in two hepatomas with rapid and intermediate growth rates. The isolated mitochondria do not show any significant differences (Kaschnitz, 1976). If this observation is confirmed, mitochondria may not be important to study in regard to malignant cell behavior.

Tumor Cell Products: Products produced by tumors and released into the serum may be useful for diagnosing cancers as well as for following the progression

of cancer therapies. Furthermore, they may be important in defining the preneoplastic state when therapies are known to be most effective. Patients with leukemias, lymphomas, and melanoma have elevated levels of a DNA-binding protein (Parsons et al., 1976; Parsons and Hoch, 1977). Histaminase has been associated with ovarian and other kinds of cancers (Lin and Kirley, 1976; Stolbach et al., 1976). Serum galactosyltransferase may prove useful in evaluating and monitoring patients with neoplastic disease (Weiser et al., 1976). Ninety percent of patients with pancreatic cancer have elevated serum ribonuclease (Reddi and Holland, 1976).

Such findings are observed in animal models and patient populations. Often these products in this serum are expressions of fetal gene activity confirming that the neoplastic state is related to embryonic development, when cells are also migrating and rapidly dividing.

Other Studies: Other developments in the Tumor Biology Program show great potential for clinical application. One of these developments is the *in vivo* synchronization of murine sarcoma ascites tumor cells using hydroxyurea (Matthews et al., 1976). Most cancer chemotherapeutic agents currently available are cell-cycle dependent. To kill a significant fraction of tumor cells in an asynchronously dividing population, chemotherapy must be maintained for a long time. This prolonged exposure is often dangerously toxic. Treating synchronous cell populations should be milder and more complete.

Tumor cells often do not take up drugs effectively, but drug-resistant tumor cells can be made sensitive when fused with drug-containing lipid vesicles (Poste and Papahadjopoulos, 1976). Possibly vesicles can be developed specifically directed via the vascular system to a given tumor. Such vesicles may improve delivery of pharmaceutical agents to tumors without endangering other cell populations.

Emphasis and Projections

The Tumor Biology Program will continue to emphasize and encourage the following research areas:

(a) Characterization of cell surface glycoproteins and glycolipids specific to tumor cells.

(b) Definition of the contribution of cancer-specific glycoproteins and glycolipids to cell movement, cell migration (invasiveness), and breakdown of specific cell-cell interactions.

(c) Determination of microtubular and microfilament involvement in the cancer-cell phenotype.

(d) Establishment of *in vitro* to *in vivo* model systems (like the B16 melanoma metastatic variants) that can be used to examine nutritional requirements of tumor cells, and which are amenable to molecular determination of specific cancer cell components that contribute to uncontrolled cell growth and uncontrolled cell movement.

(e) Further development and use of somatic cell genetics, somatic cell hybridization, and DNA recombinant research with cancer cells.

(f) Continued development of model systems to study the regulation of specific gene product synthesis.

(g) Further delineation of regulatory controls specifically involved in cell cycle growth.

(h) Expanded research to purify tumor angiogenesis factor and to clarify the mechanism of induced tumor vascularization.

Developments and improvements in the past few years of mammalian cell culture and somatic cell genetics cell systems will replace bacteria in studies related to cancer. The mammalian cell systems and model systems currently available are amenable to studies which relate directly to problems of neoplasia. No further expansion of plant tumors research will be encouraged. Expansion in this area must await evidence of high relevance to cancer.

Other Program Activities

The Tumor Biology Program encourages small rather than large conferences. This emphasis on small conferences reflects the conviction that small, focused conferences improve scientific exchange and provide better information for establishing program priorities. A number of workshops are being considered in the areas of cancer genetics, cancer cell movement and adhesion, and glycoproteins of cancer cell surfaces.

TUMOR BIOLOGY PROGRAM

SUMMARY BY SUB-CATEGORY
(Dollars in Thousands)

	NONCOMPETING NO.	NONCOMPETING AMOUNT	COMPETING NO.	COMPETING AMOUNT	TOTAL NO.	TOTAL AMOUNT
CELL SURFACE	65	$4,349	18	$1,599	83	$5,948
ENZYMES	48	2,999	8	859	56	3,858
PEPTIDE HORMONES	14	930	1	84	15	1,014
STEROIDS	13	2,969	3	297	16	3,266
MEMBRANEOUS ORGANELLES	9	765	1	44	10	809
RIBOSOMES AND POLYRIBOSOMES	5	479	3	246	8	725
M-RNA	10	847	6	424	16	1,271
T-RNA	5	223	2	166	7	389
DNA	13	786	5	272	18	1,058
GROWTH FACTORS	23	1,492	5	339	28	1,831
NUCLEUS	17	1,169	6	429	23	1,598
CONTRACTILE ELEMENTS	2	156	2	102	4	258
DEVELOPMENT AND DIFFERENTIATION	19	1,400	5	506	24	1,906
CELL GROWTH, CELL DIVISION	4	463	4	469	8	932
SOMATIC CELL GENETICS	10	815	3	208	13	1,023
INHERITANCE OF NEOPLASMS	3	177	0	0	3	177
PLASMIDS, VIRUSES	5	293	3	218	8	511
NO CLASSIFICATION	5	774	0	0	5	774
VO - PROGRAM PROJECTS	3	1,041	3	1,667	6	2,708
NOT CODED	2	55	6	312	8	367
TOTAL TUMOR BIOLOGY	275	$22,182	84	$8,241	359	$30,423

REFERENCES

Amos, H., Leventhal, M., Chu, L., and Karnovsky, M. J., Modifications of Mammalian Cell Surfaces Induced by Sugars: Scanning Electron Microscopy. Cell 7:97-103, 1976. (CA 19015)

Baserga, R., Huang, C-H., Rossini, M., Chang, H., and Ming, PM.L., Role of Nuclei and Nucleoli in the Control of Cell Proliferation. Cancer Res. 36:4297-4300, 1976. (CA 08373, CA 12923)

Brinckerhoff, C. and Lubin, M., Prolonged Inhibition of Protein and Glycoprotein Synthesis in Tumor Cells Treated with Muconomycin A. J. Natl. Cancer Inst. 58:605-609, 1976. (CA 15085, CA 17293)

Brooks, R. F., Regulation of the Fibroblast Cell Cycle by Serum. Nature 260:248-250, 1976. (CA 14195, CA 11176)

Burns, R. L., Rosenberger, P. G., and Klebe, R., Carbohydrate Preferences of Mammalian Cells. J. Cell Physiol. 88:307-316, 1976. (CA 19017)

Chiu, J-F. and Hnilica, L. S., DNA Polymerases in Developing and Neoplastic Tissues. Oncodevelopmental Gene Expression, Academic Press, New York, 65-74, 1976. (CA 18389)

Fenselau, A. and Mello, R. J., Growth Stimulation of Cultured Endothelial Cells by Tumor Cell Homogenates. Cancer Res. 36:3269-3273, 1976. (CA 15381)

Goldstein, L., Role for Small Nuclear RNA's in "Programming" Chromosomal Information. Nature 261:519-521, 1976. (CA 13419)

Hankinson, O., Mutants of the Chinese Hamster Ovary Cell Line Requiring Alanine and Glutamate. Somatic Cell Genetics 2:497-507, 1976. (CA 15794, CA 13419)

Hynes, R. O., Cell Surface Proteins and Malignant Transformation. Biochimica et Biophysica Acta 458:73-107, 1976. (CA 17007)

Hynes, R. O. and Pearlstein, E. S., Investigations of the Possible Role of Proteases in Altering Surface Proteins of Virally Transformed Hamster Fibroblasts. J. Supramolecular Structure 4:1:1-14, 1976. (CA 17007)

Itaya, K., Hakomori, S-I., and Klein, G., Long Chain Neutral Glycolipids and Gangliosides of Murine Fibroblast Lines of Low- and High-Tumorigenic Hybrids. Proc. Natl. Acad. Sci. 73:1568-1571, 1976. (CA 20026, CA 14054)

Johnson, R. K., Inouye, T., Goldin, A., and Stark, G. R., Antitumor Activity of N-Phosphonacetyl-L-Aspartate, A Transition State Analog Inhibitor of Aspartate Transcarbamylase. Cancer Res. 36:2720, 1976. (CA 17287)

Kaschnitz, R. M., Hatefi, Y., and Morris, H. P., Oxidative Phosphorylation Properties of Mitochondria Isolated from Transplanted Hepatoma. Biochemica et Biophysica Acta 449:224-235, 1976. (CA 13609)

Kempe, T. D., Swyryd, E. A., Bruist, M., and Stark, G. R., Stable Mutants of Mammalian Cells that Overproduce the First Three Enzymes of Pyrimidine Nucleotide Biosynthesis. Cell 9:627, 1976. (CA 17287)

Klebe, R. J., Rosenberger, P. G., Naylor, S. L., Burns, R. L., Novak, R., and Kleinman, H., Cell Attachment to Collagen. Exp. Cell Res. 104: 119-125, 1977. (CA 19017)

Knowles, B. B., Solter, D., Trinchieri, G., Maloney, K. M., Ford, S. R., and Aden, D. P., Complement-Mediated Antiserum Cytotoxic to Human Chromosome 7 Coded Antigen(s): Immunoselection of Rearranged Chromosome 7 in Human-Mouse Somatic Cell Hybrids. J. Exp. Med. 145:314-326, 1977. (CA 10815, CA 17540, CA 18470)

Lin, C. W. and Kirley, S. D., Histaminase as an Onco-Placental Enzyme: Biochemical and Immunological Evidence. Onco-Developmental Gene Expression, Academic Press, New York, Ch. 15, 1976. (CA 17194)

Lipkin, G. and Knecht, M. E., Contact Inhibition of Growth is Restored to Malignant Melanocytes of Man and Mouse by a Hamster Protein. Exp. Cell Res. 102:341-348, 1976. (CA 16013)

Lipkin, G. Rosenberg, M., and Knecht, M. E., Factors Affecting Growth of Normal and Malignant Cells In Vitro. Biochemical Pharm. 25: 1333-1337, 1976. (CA 16013, CA 18034)

Matthews, R. H., Dewar, D., and Webb, T. E., In Vivo Synchronization of Murine Sarcoma 37 Ascites Tumor Cells by Use of Hydroxyurea. Physiol. Chem. and Physics 8:151-155, 1976. (CA 12411)

Messmer, T. O. and Young, D. V., Effects of Biotin and Fatty Acids on SV3T3 Cell Growth in the Presence of Normal Calf Serum. J. Cell Physiol. 90:265-270, 1976. (CA 20040, CA 11176)

Ming, P-M.L., Chang, H. L., and Baserga, R., Release by Human Chromosome 3 of the Block at G, of the Cell Cycle, in Hybrids Between ts AF8 Hamster and Human Cells. Proc. Natl. Acad. Sci. 73:2052-2055, 1976. (CA 08373, CA 12923)

Monohan, T. M., Fritz, R. R., and Abell, C. W., Serum Factor: The Inhibition of Phytohemagglutinin-Induced Blastogenesis of Chronic Lymphocytic Leukemia Lymphocytes. J. Natl. Cancer Inst. 57:217-220, 1976. (CA 14525)

Muramatsu, T., Koide, N., Ceccarini, C., and Atkinson, P. H., Characterization of Mannose-labeled Glycopeptides from Human Diploid Cells and Their Growth-dependent Alterations. J. Biol. Chem. 251: 4673-4679, 1976. (CA 13402, CA 06576)

Naylor, S., Busby, L. L., and Klebe, R. J., Biochemical Selection Systems for Mammalian Cells: The Essential Amino Acids. Somatic Cell Genetics 2:93-111, 1976. (CA 19017)

Nicolson, G. L., Transmembrane Control of the Receptors on Normal and Tumor Cells, I. Cytoplasmic Influence Over Surface Components. Biochimica et Biophysica Acta 457:57-108, 1976a. (CA 15122)

Nicolson, G. L., Transmembrane Control of the Receptors on Normal and Tumor Cells, II. Surface Changes Associated with Transformation and Malignancy. Biochimica et Biophysica Acta 458:1-72, 1976b. (CA 15122)

Nicholson, G. L., Winkelhake, J. L., and Nussey, A. C., An Approach to Studying the Cellular Properties Associated with Metastasis: Some In Vitro Properties of Tumor Variants Selected In Vivo for Enhanced Metastasis. North-Holland Publishing Co., Ch. 15, 1976. (CA 15122)

Parsons, R. G. and Hoch, J. A., Purification and Identification of a Human-Serum DNA-Binding Protein Associated with Malignant Diseases. Eur. J. Biochem. 71:1-8, 1976. (CA 17563)

Parsons, R. G., Longmere, R. L., Hoch, S. O., and Hoch, J. A., A Clinical Evaluation of Serum C3DP Levels in Individuals with Malignant Diseases. Cancer Res. 37:692-695, 1977. (CA 13465, CA 17563)

Pearlstein, E., Hynes, R. O., Franks, L. M., and Hemmings, V. J., Surface Proteins and Fibrinolytic Activity of Cultured Mammalian Cells. Cancer Res. 36:1475-1480, 1976. (CA 17007)

Poste, G. and Papahadjopoulos, D., Drug-containing Lipid Vesicles Render Drug-Resistant Tumor Cells Sensitive to Actinomycin D. Nature 261:699-701, 1976. (CA 13393, CA 18372, CA 18260)

Ramanarayanan-Murthy, L., Colman, P. D., Morris, H. P., and Feigelson, P., Pretranslational Control of Tryptophan Oxygenase levels in Morris Hepatoma and Host Liver. Cancer Res. 36:3594-3599, 1976. (CA 02332, CA 10729)

Reddi, K. K. and Holland, J. F., Elevated Serum Ribonuclease in Patients with Pancreatic Cancer. Proc. Natl. Acad. Sci. 73:2308-2310, 1976. (CA 15936)

Rose, K. M. and Jacob, S. T., Nuclear Poly (A) Polymerase from Rat Liver and a Hepatoma. Eur. J. Biochem. 67:11-21, 1976. (CA 16438, CA 15733)

Rose, K. M., Ruch, P. A., Morris, H. P., and Jacob. S. T., RNA Polymerases from a Rat Hepatoma. Biochemica et Biophysica Acta 432:60-72, 1976. (CA 16438)

Scott, R. E. and Furcht, L. T., Membrane Pathology of Normal and Malignant Cells--A Review. Human Pathology 7:519-532, 1976. (CA 16228)

Shubik, P., Feldman, R., Garcia, H., and Warren, B. A., Vascularization Induced in the Cheek Pouch of the Syrian Hamster by Tumor and Nontumor Substances. J. Natl. Cancer Inst. 57:769-774, 1976. (CA 14766)

Stanbridge, E. J., Suppression of Malignancy in Human Cells. Nature 260:17-20, 1976. (CA 19401)

Stolbach, L., Inglis, N., Lin, C., Turksoy, R. N., Fishman, W., Marchant, D., and Rule, A., Measurement of Regan Isoenzyme, HCG, CEA, and Histaminase in the Serum and Effusion Fluids of Patients with Carcinoma of the Breast, Ovary, or Lung, Onco-Developmental Gene Expression. Academic Press, New York, Ch. 49, 1976. (CA 17194)

Swift, M., Malignant Disease in Heterozygous Carriers, Birth Defects: Original Article Series. Volume XII, Number I., National Science Foundation. 133-144, 1976. (CA 15207)

Watanabe, K., Matsubara, T., and Hakomori, S-H., αL-Fucopysanosylceramide, A Novel Glycolipid Accumulated in Some of the Human Colon Tumors. J. Biol. Chem. 251:2385-2387, 1976. (CA 10909, CA 12710)

Weiser, M., Podolsky, D., and Isselbacker, K., Cancer-Associated Isoenzyme of Serum Galactosyltransferase. Proc. Natl. Acad. Sci. 73:1319-1322, 1976. (CA 16703)

Whelly, S. and Baserga, R., Relationship Between Cell Proliferation, Chromatin Template Activity and Accumulation of Nuclear Proteins. Cell Biology International Reports, 1:13-21, 1977. (CA 08373)

Yeoman, L., Seeber, S., Taylor, C., Fernbach, D., Falletta, J., Jordan, J., and Busch, H., Differences in Chromatin Proteins of Resting and Growing Lymphocytes. Exp. Cell Res. 100:47-55, 1976. (CA 10893)

IMMUNOLOGY PROGRAM

Description

The Immunology Program is focused on the development of effective immunologic approaches to cancer prevention, diagnosis, and treatment. Because these approaches depend to a large extent upon an understanding of tumor antigens and the mechanisms of immune reactions, much of the program emphasis is on basic immunology.

Both basic and clinical research are supported in the following areas: cellular immunology; identification, isolation, and characterization of cell surface antigens; antibody structure, synthesis, and function; humoral factors involved in immunologic reactions; immune status; immunogenetics; immunoprophylaxis against cancer; immunodiagnostic tests for cancer; animal and *in vitro* studies of cancer immunotherapy; and research studies of clinical cancer immunotherapy.

Accomplishments

Major advances have been made in the past year toward understanding immunogenetics in relation to cancer. Genetic control of responses to tumor specific antigens has been demonstrated, and some background knowledge has developed about genetic restrictions of immunologic responsiveness. Evidence has also accumulated suggesting that some tumor antigens may represent modified histocompatibility antigens; while not yet conclusive, this evidence has opened a major new approach to the search for human tumor antigens.

An intriguing relationship between histocompatibility type and mating preference, apparently mediated by scent, was demonstrated in animals. This observation may seem remote to cancer, but if tumor antigens are modified histocompatibility antigens, it is not inconceivable that cancer diagnostic tests or perhaps even a specific therapy might be devised based upon detection of altered pheromones.

New immunologic techniques made it possible to separate subpopulations of lymphocytes and to correlate their functions with specific cell surface markers. As a result, control of differentiation and function of subpopulations of immune cells is currently the subject of intensive investigation. It is now possible for the first time to distinguish serologically between lymphocytes which can kill target cells, those which help in killing, and those which suppress immune reactions.

The demonstration that antigens which distinguish between the two major classes of lymphocytes (T and B cells) exist in man as well as in mice is another major advance. Antisera have been developed to distinguish between T and B cell leukemias, and it has already been demonstrated that this distinction is important in relation to appropriate chemotherapy.

Improved technology permitted isolation of immune cells from tumor masses in sufficient quantities and with adequate viability to permit studying the

functional characteristics of cells which have been in close contact in vivo with tumor cells. Methods for sensitizing human lymphocytes in vitro have also been developed, with the ultimate goal of returning these sensitized cells to the patient to act against his own tumor.

A newly recognized "natural killer" cell (NK cell) reacts to a variety of tumor cells in vitro without previous known exposure of the donor to the tumors being tested. Several laboratories are investigating the possibility that this type of cell plays a role in a previously unrecognized surveillance against tumor development and progression.

Regional immunotherapy for lung cancer using intrapleural BCG is giving encouraging results, and clinical trials are being expanded. Promising preliminary studies have also been reported for combined adjuvant and specific active immunotherapy for melanoma, lung cancer, and leukemia, with immunotherapy used as an adjunct to chemotherapy or surgery.

A modified CEA radioimmunoassay which may be more reliable than current assays has been devised and is being extensively tested clinically. A new immunodiagnostic test for Hodgkin's disease has also been developed and is being studied in a large patient population. Another potentially useful immunodiagnostic test has been devised for melanoma. This utilizes antisera made in nonhuman primates and is being prepared for clinical testing.

Published research accomplishments by grantees in the Immunology Program during 1976 included the following:

Drs. Cantor, Shen, and Boyse investigated whether T lymphocytes mediating specific helper and suppressor activities belong to different subclasses. Cells of the Ly 1 subclass generate helper activity in both primary and secondary responses to sheep red blood cells. In contrast, Ly 2 and especially Ly 2,3 cells had suppressive activity (Cantor et al., 1976).

Dr. Jandinski and co-workers studied functional activities of peripheral T lymphocytes subclassified on the basis of differential expression of Ly surface components. Using concanavalin A, a nonspecific polyclonal activator of T lymphocytes, these investigators demonstrated that helper and suppressor functions are manifestations of separate T cell differentiative pathways and do not depend upon stimulation of the cells by antigen (Jandinski et al., 1976).

Drs. Dennert and De Rose established in culture continuously proliferating T cells sensitized to alloantigens. Survival in culture for more than nine months was reported, and, for these cells, the antigen requirements for proliferation differed from those for cell-mediated cytotoxicity (Dennert and De Rose et al., 1976).

Dr. Burakoff and co-workers investigated functional aspects of the interaction between effector and target cells in a modified syngeneic system. Inhibition of cytolysis by antisera to K and D specificities confirmed the significance of the H-2 complex in this interaction (Burakoff et al., 1976).

A cytotoxic autologous lymphocyte response was produced in vitro against cells from a patient with acute myelomonocytic leukemia (AMML) when alogeneic stimulating cells were used to generate a strong proliferation-inducing signal. The relation between the target antigens detected on AMML cells by cell-mediated cytotoxicity and antigens detected serologically on AMML cells is presently under investigation (Zarling et al., 1976).

A model of B lymphocyte differentiation was derived from in vitro cell surface antigen induction studies. It proposed that B precursor cells pass through a minimum of four developmental compartments before becoming mature B cells. The rapidity with which new surface markers appear during induction was taken as an indication that cells in bone marrow and spleen capable of making the transition pre-exist in a poised state, each genetically programmed to express (when induced by an inductive stimulus) only the phenotype of the next compartment in sequence (Hammerling et al., 1976).

Dr. Unanue and co-workers demonstrated that mediators secreted by macrophages can alter lymphocyte responses. The secretion of lymphostimulatory molecules was shown to be controlled by extracellular factors--a particle that is phagocytosed or an activated T cell (Unanue et al., 1976).

By the use of microsequencing techniques, the amino terminal sequences of two HLA heavy chain preparators carrying different HLA allospecificities (A2 and B7,14) were determined through position 22, and identity was found at all but one position. Considerable homology was also detected with mouse H-2 antigens. The data support the hypothesis that HLA and B genes evolved from a common ancestral gene (Appella et al., 1976).

The distribution of immune-associated (Ia) antigens on spleen lymphocytes was investigated by an ultrastructural method employing deep-freeze-etched replicas. Ia antigens were detected predominantly on immunoglobulin-bearing B lymphocytes. Capping of immunoglobulin left Ia antigens diffusely distributed on the cell surface, indicating that the two are not linked (Abbas et al., 1976).

Hyperimmune antisera were used against a teratoma-derived endodermal mouse carcinoma to define a cell surface antigen. The antigen was on all tumor cells tested which were of endodermal origin, as well as on embryonic liver. Further testing revealed that the antigen was on several inappropriate cell types, such as young embryos before the appearance of endoderm, sperm, and a small proportion of nonendodermal tumors and that it must therefore be considered a "quasi-endodermal" antigen (Artzt et al., 1976).

Immunoprecipitation of radioactivity from labeled cell membrane preparations was used to study the association between the H-2 peptides and a tumor-associated embryonic antigen (Agl) on the surface of L-cells, testing the hypothesis that Agl represents an altered H-2 peptide expressed on transformed cells. Electrophoresis of Agl showed two major peaks, one co-migrating with the major H-2 peptide. Sequential immunoprecipitation indicated that the molecules reacting with antisera to H-2 and to Agl were separate entities, precipitating independently of one another. It was

postulated that Agl and H-2 participate in a unique kind of association in the cell membrane and that the bonds between them which allow them to co-cap may be in the lipid bilayer itself (Gooding et al., 1976).

The complete covalent structure for a human myeloma IgA1 immunoglobulin was determined. This protein has unique features in the amino acid sequence and the disulfide bridge structure of the variable (V) and constant (C) regions of both the α heavy and the λ light chains and in the number of oligosaccharides (Liu et al., 1976).

A detailed analysis was made of the role of the human complement system in mediating the lysis of diverse oncornaviruses in the apparent absence of antibody. The C1g subunit of the first human complement component provides a specific recognition function which enables the complement system to act as a natural defense mechanism against oncornavirus infection and replication in man (Cooper et al., 1976).

The human chromosome 7, when present in human-mouse somatic cell hybrids, codes for a surface antigen(s). The surface antigen is immunogenic in mice syngeneic to the mouse parental cell. The methodology could be used to determine the genetic control of many cell surface antigens expressed on both normal and transformed human cells (Aden and Knowles, 1976).

The expression of an oncodevelopmental protein, α1 fetoprotein (AFP), was systematically studied during normal development and in various experimental systems. Production of AFP is associated with normal hepatocyte division in both fetal and adult rats and with exposure to chemical carcinogens in the presence or absence of hepatocyte proliferation (Sell et al., 1976).

Because adjuvants enhance antibody production, but at the same time paradoxically depress cytolytic activity, the possibility that adjuvant might preferentially potentiate different T cell subpopulations was investigated. Complete Freund's adjuvant suppressed the generation of cytolytic T cells *in vivo* when mixed with allogeneic P815 cells before immunization of C57BL/6 mice. Memory T cells, however, were not subject to the same suppressive regulation as were precytotoxic T cells since adjuvant had no effect on subsequent boosting of memory *in vitro* (Reinisch et al., 1976).

Dr. Weiden and co-workers have provided evidence that a combination of procarbazine and antithymocyte serum can abrogate sensitization to histocompatibility antigens induced by multiple prior transfusions in histocompatible unrelated canine donor-recipient pairs, and thus permit successful engraftment of donor bone marrow. Studies are now under way in human patients with aplastic anemia using the same regimen as in the canine study (Weiden et al., 1976).

Host immunity in the response to chemotherapy of the highly immunogenic mouse mammary adenocarcinoma T1699 was investigated. Suppression of the host response by previous irradiation or antilymphocyte serum decreased the effect of both cyclophosphamide and melphalan, while melphalan given seven days after tumor induction in nonimmunosuppressed mice proved ineffective.

Host effector cells found within the tumor itself had significant colony-inhibiting activity, whether or not the animal had been given a chemotherapeutic agent (Radov et al., 1976).

A specific interaction between serum factors and the surface of peripheral T lymphocytes from patients with Hodgkin's disease may provide the basis for a diagnostic test. In a study of the defective binding of sheep erythrocytes to the surface of peripheral blood T lymphocytes (E-rosette formation) in Hodgkin's disease, the decreased percentage of E-rosette-forming cells could be reversed by prior incubation in tissue-culture medium and fetal calf serum. This reversal could be suppressed by additional incubation with serum from patients with Hodgkin's disease (Fuks, et al., 1976).

The therapeutic efficacy of BCG in malignant melanoma was evaluated. Five of fifteen patients studied showed significant objective improvement; all of these were patients with dermal metastatic disease. In vitro cytotoxicity in the presence of the patient's serum, bore, on average, a relationship to the clinical disease. In certain cases serum blocking and/or lymphocyte stimulation may have prognostic significance (Mastrangelo et al., 1976).

Sixty lung cancer patients were studied in a randomized prospective trial of regional therapy with intrapleural BCG. Forty patients were followed for more than a year. BCG had improved survival in those patients with a limited tumor burden. Intrapleural BCG did not seem beneficial in patients with advanced disease (McKneally et al., 1976).

Emphases and Projections

Immunobiology: Rapid advances in the understanding of immunologic mechanisms can be expected now that sophisticated analyses of the functional characteristics of subpopulations of lymphocytes are possible. A substantial amount of immunobiologic research specifically directed at cancer is developing and will expand. Growing areas of interest include: research on improved methodology for studies of cell-mediated immunity; further analyses of subclasses of lymphocytes and use of cell-surface markers to resolve serological plus functional heterogeneity; studies of possible structural and functional relationships between tumor antigens and histocompatibility antigens; studies of natural killer (NK) cells; investigations of genetic control of immunologic responses to tumor antigens; studies of mediators produced by tumors which affect host mechanisms; research on the relationship between malignancy and antigens related to blood group substances; studies of anti-idiotype antisera for manipulating specific immune responses; and studies of regulating immune responses by cellular interactions and by regulatory factors produced by lymphocytes.

Certain other areas of cancer-relevant immunobiology remain relatively underdeveloped and require continued stimulation. One such area is the identification and characterization of new human tumor antigens which is fundamental to developing new immunodiagnostic tests and specific immunotherapy. Encouragement is also given to new studies on immunogenicity of

spontaneous tumors, manipulation of immune responses to promote tumor rejection, spontaneous killing of human target cells by normal lymphocytes, mechanisms of resistance to bone marrow transplants, polygenic factors affecting host immunologic resistance to tumors, and mechanisms by which tumors escape host immunologic defenses.

Immunodiagnosis: Several promising new approaches to immunodiagnosis will result in expanded research. Altering the level of natural immunity toward the Thomsen-Friedenreich (T) blood group antigen shows promise as a diagnostic test for human breast cancer, and clinical studies of its usefulness as a diagnostic and prognostic aid are being initiated. Testing is also under way of a promising immunodiagnostic test for Hodgkin's lymphoma and of a potentially useful test for melanoma which utilizes antisera made in nonhuman primates. Major emphasis will continue on isolation and characterization of new human antigens for cancer-specific immunodiagnostic tests.

Immunotherapy: Studies of mechanisms of actions of immunostimulants and animal-model studies of new approaches to immunotherapy, particularly in combination with other treatments, are currently emphasized. Several clinical trials of immunotherapy are under way, including a study of non-Hodgkin's lymphoma with interferon. Promising early results of adjuvants combined with specific active immunotherapy suggest possible expansion of clinical trials of immunotherapy combined with other modalities.

Immunology in Relation to Cause and Prevention: The relation of the immune response to the origin and development of cancer remains controversial. A substantial amount of research will continue in this area for some years. A large-scale investigation of effects of immune responses on tumor-cell populations was recently initiated. A major expansion of projects exploring the interface between immunologic responses and carcinogenesis is anticipated.

The recent demonstration that NK cells can kill tumor cells without prior sensitization has opened another approach to studying immune surveillance against cancer. Extensive new studies will investigate the properties of these cells which differ from both T and B cells.

Other Program Activities

Attracting capable young immunologists into cancer-relevant research continues to have high priority. A meeting of young investigators with leaders in tumor immunology was held in Bar Harbor, Maine, in September 1976. This was the third small regional meeting of this kind, and it was well received by the young investigators. Senior immunologists who have participated in organizing and conducting these conferences have uniformly expressed enthusiasm and recommend continuation of this effort.

Close coordination is maintained with NCI's intramural and contract immunology programs and with the immunology programs of the National Institute of Allergy and Infectious Diseases. This takes the form of participation in meetings of contract committees when program planning is involved. Exchange of information also occurs with program staff at the American Cancer Society and the National Science Foundation.

A conference on relationships between the immunologic state and disease will be cosponsored by the National Institute of Allergy and Infectious Diseases and the National Cancer Institute. This conference is now in the planning stage, as is a workshop on immunologic responsiveness of cancer patients. Immunology meetings partially supported during the past year included a workshop on immunology in relation to genitourinary tract cancer, a workshop on brain tumor immunology, a Gordon Conference on Tumor Immunology, the Sixth International Transplantation Congress, and the Third International Congress of Immunology.

Among those providing advice to the Immunology Program, DCRRC, were: Drs. D. Bernard Amos, Baruj Benacerraf, Edward A. Boyse, Fritz Bach, Max D. Cooper, Edward S. Golub, Harold E. Dvorak, Robert A. Good, Ingegerd E. Hellstrom, Christopher S. Henney, Evan M. Hersh, Sherwood H. Lawrence, Frank Lilly, Donald Morton, Lloyd Old, Richmond T. Prehn, David Pressman, George W. Santos, Stewart Sell, Osias Stutman, Charles W. Todd, Darcy B. Wilson, and Henry J. Winn.

IMMUNOLOGY PROGRAM

SUMMARY BY SUB-CATEGORY
(Dollars in Thousands)

	NONCOMPETING		COMPETING		TOTAL	
	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT
ANTIBODY STRUCTURE, SYNTHESIS AND FUNCTION	19	$1,352	9	$899	28	$2,251
IDENTIFICATION OF CELL SURFACE ANTIGENS	46	3,717	26	1,915	72	5,632
CELLULAR IMMUNOLOGY	87	10,865	22	2,046	109	12,911
DIAGNOSTIC TESTS	1	70	0	0	1	70
HUMORAL FACTORS OTHER THAN ANTIBODY	17	1,018	2	117	19	1,135
IMMUNODIAGNOSTIC TESTS FOR CANCER	3	260	1	117	4	377
IMMUNOGENETICS	25	1,546	15	1,332	40	2,878
IMMUNOPROPHYLAXIS	1	51	0	0	1	51
IMMUNE STATUS	30	3,034	2	205	32	3,239
IMMUNOTHERAPY ANIMAL MODELS IN VITRO STUDIES	20	1,645	6	411	26	2,056
CLINICAL IMMUNOTHERAPY	8	2,980	3	1,116	11	4,096
MECHANISMS FOR IMMUNOTHERAPY	15	1,076	2	141	17	1,217
TOTAL	272	$27,614	88	$8,299	360	$35,913

REFERENCES

Abbas, A. K., Dorf, M. E., Karnovsky, M. J., and Unanue, E. R., The Distribution of Ia Antigens on the Surfaces of Lymphocytes. J. Immunol. 116:371-378, 1976. (CA 14723)

Aden, D. P. and Knowles, B. B., Cell Surface Antigens Coded for by the Human Chromosome. Immunogenetics 3:209-221, 1976. (CA 18470)

Appella, E., Tanigaki, N., Gairwell, T., and Pressman, D., Partial Amino Acids Sequences of the Heavy Chains of Human HLA Histocompatibility Antigens. Biochem. Biophy. Res. Comm. 71:286-292, 1976. (CA 17276)

Artzt, K., Hamburger, L., Jakob, H., and Jacob, F., Embryonic Surface Antigens: A Quasi-Endodermal Teratoma Antigen. Dev. Biol. 51:152-157, 1976. (CA 21651)

Burakoff, S., Germain, R., Dorf, M., and Benacerraf, B., Inhibition of Cell-Mediated Cytolysis of Trinitrophenyl-Derivatized Target Cells by Alloantisera Directed to the Products of the K and D Loci of the H-2 Complex. Proc. Nat. Acad. Sci. USA 73:625-629, 1976. (CA 14723)

Cantor, H., Shen, F. W., and Boyse, E. A., Separation of Helper T Cells from Suppressor T Cells Expressing Different Ly Components. J. Exp. Med. 143:1391-1401, 1976. (CA 16889)

Cooper, N. R., Jensen, F. C., Welsh, R. M., Jr., and Oldstone, M. B. A., Lysis of RNA Tumor Viruses by Human Serum: Direct Antibody-Independent Triggering of the Classical Complement Pathway. J. Exp. Med. 144: 970-984, 1976. (CA 14692)

Dennert, G. and De Rose, M., Continuously Proliferating T Killer Cells Specific for H-2^b Targets: Selection and Characterization. J. Immunol. 116:1601-1602, 1976. (CA 15581)

Fuks, Z., Strober, S., and Kaplan, H. S., Interaction Between Serum Factors and T Lymphocytes in Hodgkin's Disease. J. Medicine 295:1273-1278, 1976. (CA 17004)

Gooding, L., Expression of Early Fetal Antigens on Transformed Mouse Cells. Cancer Res. 36:3499-3502, 1976. (CA 16802)

Hammerling, U., Chin, A. F., and Abbott, J., Ontogeny of Murine B Lymphocytes: Sequence of B-Cell Differentiation from Surface-Immunoglobulin-Negative Precursors to Plasma Cells. Proc. Natl. Acad. Sci. USA 73:2008-2011, 1976.

Jandinski, J., Cantor, H., Tadakuma, T., Peavy, D. L., and Pierce, C. W., Separation of Helper T Cells from Suppressor T Cells Expressing Different Ly Components. J. Exp. Med. 143:1382-1390, 1976. (CA 14723)

Liu, Y. V., Low, T. L. K., Infante, A., and Putnam, F. W., Complete Covalent Structure of a Human IgA1 Immunoglobulin. Science 193:1017-1020, 1976. (CA 08497)

Mastrangelo, M. J., Sulit, H. L., Prehn (Melartin), L., Bornstein, R. S., Yarbro, J. W., and Prehn, R. T., Intralesional BCG in the Treatment of Metastatic Malignant Melanoma. Cancer 37:684-692, 1976. (CA 13456)

McKneally, V. F., Maver, C., and Kausel, H. W., Regional Immunotherapy of Lung Cancer with Intrapleural BCG. The Lancet 377-379, 1976. (CA 17346)

Radov, L. A., Haskill, J. S., and Korn, J. H., Host Immune Potentiation of Drug Responses to a Murine Mammary Adenocarcinoma. Int. J. Cancer 17:773-779, 1976. (CA 17694)

Reinisch, C. L., Gleiner, N. A., and Schlossman, S. F., Adjuvant Regulation of T Cell Function. J. Immunol. 116:710-715, 1976. (CA 15679)

Sell, S., Becker, F. F., Leffert, H. L., and Watabe, H., Expression of an Oncodevelopmental Gene Product (α-Fetoprotein) During Fetal Development and Adult Oncogenesis. Cancer Res. 36:4239-4249, 1976. (CA 14312)

Unanue, E. R., Beller, D. I., Calderon, J., Kiely, J. M., and Stadecker, M. J., Regulation of Immunity and Inflammation by Mediators from Macrophages. Am. J. Pathol. 85:465-478, 1976. (CA 14723)

Weiden, P. L., Storb, R., Slichter, S., Warren, R. P., and Sale, G. E., Effect of Six Weekly Transfusions on Canine Marrow Grafts: Tests for Sensitization and Abrogation of Sensitization by Procarbazine and Antithymocyte Serum. J. Immunol. 117:143-150, 1976. (CA 18047)

Zarling, J. M., Raich, P. C., McKeough, M., and Bach, F. H., Generation of Cytotoxic Lymphocytes In Vitro Against Autologous Human Leukemic Cells. Nature 262:691-693, 1976.

CARCINOGENESIS PROGRAM

Description

The Carcinogenesis Program supports a broad range of studies collectively concerned with achieving more effective prevention of human cancer, caused or promoted by chemical or physical agents, or by the interaction of these with biological agents. The program includes two complementary categories of research: Chemical Carcinogenesis and Cancer Biochemistry.

In Chemical Carcinogenesis the emphasis is on mechanisms of action; the stepwise changes by which a cancer-producing chemical or physical agent affects the transformation of normal cells to the cancerous state. The principal kinds of research included here are studies on: (a) molecular structure-carcinogenicity relationships, identification of carcinogens and their metabolites, and development of analytical procedures applicable to body fluids, tissues, and environmental specimens; (b) biochemical changes in physiological compounds and processes produced by chemical and physical carcinogens; (c) properties of cells transformed by chemical carcinogens; (d) metabolism of chemical carcinogens, identification of proximate and ultimate carcinogens, and carcinogenicity-mutagenicity relationships; (e) DNA damage by chemical and physical carcinogens and the nature and extent of the subsequent DNA repair; (f) inhibition of carcinogenesis by dietary, hormonal, or other means; (g) the role of diet and nutrition in cancer; and (h) evaluation of the importance of promoters in environmental carcinogenesis. In summary, Chemical Carcinogenesis encompasses studies generally referred to as environmental carcinogenesis research.

In Cancer Biochemistry the focus is on biochemical aspects of cancer cells and of cancerous hosts, that are significantly related to Chemical Carcinogenesis. The emphasis is on: (a) endocrine-related biochemistry; e.g., role of hormones in breast cancer and studies on hormone sensitive tumors; (b) comparisons of normal and neoplastic tissues with respect to selected enzyme systems, activities, and associated inhibitors; e.g., aryl hydrocarbon hydroxylase, proteases, epoxide hydrase and microsomal mixed function oxidases; and (c) development of biochemical methods for early detection of cancer.

Research Accomplishments

Chemical Carcinogenesis: Polycyclic aromatic hydrocarbons are an important class of environmental carcinogens which must be metabolically activated to exert their carcinogenic effects. Recent evidence indicates (Marquardt et al., 1976) that the simple K-region epoxides which are their initial metabolic derivatives are probably not involved in the carcinogenic activity of such hydrocarbons as 7-methylbenz(a)anthracene, 7,12-dimethylbenz(a)-anthracene, and benzo(a)pyrene. Another type of derivative, non-K-region dihydrodiol, can be further metabolized to diol epoxides which react in vitro with DNA to yield products resembling those found in hydrocarbon-treated cells. Several diol epoxides are more active than the parent

hydrocarbons in inducing malignant transformation of mouse fibroblasts. Transformed, morphologically abnormal cells injected into isologous mice develop into sarcomas. Thus available evidence suggests that metabolic activation of at least some polycyclic hydrocarbons proceeds via diol epoxide formation but, in most cases, the specific diol epoxides have not been identified.

One of the most extensively studied hydrocarbons is benzo(a)pyrene (BP). Evidence has been obtained (Weinstein et al., 1976) that a specific isomer of a diol epoxide derivative of BP, (±)-7β,8α-dihydroxy-9α, 10α-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene is an intermediate in binding BP to RNA in cultured bovine bronchial mucosa. An adduct is formed between position 10 of this derivative and the 2-amino group of RNA guanine. How such a hydrocarbon-nucleoside adduct might distort the conformation and function of the modified nucleic acid remains to be determined.

The known hydrocarbon content of cigarette smoke condensate appears inadequate to account for its observed carcinogenicity. An epidemiologic study (Hammond et al., 1969) indicates, for example, that roofing workers, who are exposed to enormously greater amounts of BP than are heavy cigarette smokers, have lung cancer death rates not significantly higher than the average U.S. population. Two recent studies, therefore, sought to identify tobacco smoke components which might enhance hydrocarbon carcinogenicity.

In the first of these studies (Van Duuren and Goldschmidt, 1976), a series of 21 tobacco-smoke components and related compounds were tested for their ability to enhance the activity of the carcinogens in the smoke condensate. The test compounds were applied to mouse skin three times weekly for periods exceeding one year; each application also contained a low dose (5μg) of BP. Carcinogenicity of the latter was remarkably enhanced by catechol, pyrogallol, decane, undecane, pyrene, benzo(e)pyrene, and fluoranthene. Several other compounds diminished the carcinogenicity of BP, and four inhibited it completely--esculin, quercetin, squalene, and oleic acid. Understanding the relationship between the chemical structures of these compounds and their enhancing or inhibitory activities should contribute to the development of a less hazardous cigarette.

Because tobacco smoke contains known carcinogens in addition to hydrocarbons, the possibility of synergism was investigated (Argus and Arcos, 1976). Typical microsomal enzyme inducers such as 3-methylcholanthrene (MC) inhibit the hepatocarcinogenicity of nitrosamines by repressing synthesis of enzymes which transform these compounds to proximate carcinogens. Unexpectedly, although MC depressed the hepatocarcinogenicity of dimethylnitrosamine (DMN), the two agents had a strong synergistic effect in lung tumor induction in rats. Indeed, whereas at the levels used in the study neither the nitrosamine nor the hydrocarbon alone was carcinogenic in the lung, combined administration of the two agents brought about a 15 percent pulmonary tumor incidence. Two alternative mechanisms are proposed to explain this "synergism." One possibility is that MC is responsible for tumorigenesis and that DMN increases the topically available hydrocarbon epoxide (arene oxide), perhaps by lowering the level of inactivating epoxide hydrases.

Alternatively, because they inhibit hepatic metabolism, more carcinogen might be available for tumorigenesis in extrahepatic tissues such as the lung. The practical significance of DMN and MC "synergism" in the lung is that (a) MC is a carcinogen very similar in action to 3,4-benzopyrene and 3,4-benzofluoranthene known to be present in cigarette smoke and (b) DMN, methylethylnitrosamine, and N-nitrosopiperidine are also present in the smoke. It is, therefore, highly probable that a substantial proportion of the lung tumor incidence of smokers is due to synergism between carcinogenic hydrocarbons and nitrosamines present in the smoke, rather than to the hydrocarbons alone. In addition to the carcinogenic hydrocarbons and nitrosamines present in the smoke, diets rich in nitrosamine contaminants or pre-nitrosamine components (i.e., nitrite plus secondary amines) may contribute to lung cancer induction. The lung is, in fact, a target tissue in experimental carcinogenesis by orally administered DMN (Argus and Hoch-Ligeti, 1961).

The toxicity, carcinogenicity, and mutagenicity of arene oxides of polycyclic aromatic hydrocarbons appear to correlate well with the extent of their binding to macromolecules within susceptible cells. Damage to DNA isolated from human neonatal and fetal skin cells was measured after exposure to 3-methylcholanthrene (3-MC) and its 11,12-oxide (Vaught et al., 1976). Three-methylcholanthrene itself did not induce single-strand breaks in the human cell DNA but its 11,12-oxide was very effective in this regard. The breaks occurred during a 60-minute incubation period and were repaired during the following 60 minutes.

Binding of benzo(a)pyrene (BP) metabolites to nuclear DNA, nuclear proteins, and microsomal proteins was observed in another study (Vaught and Bresnick, 1976), which confirmed the existence of an inducible nuclear aryl hydrocarbon hydroxylase (AHH) in rat liver. BP metabolism was formerly thought to depend solely on microsomal AHH. When both nuclei and microsomes used in the *in vitro* system were obtained from 3-methylcholanthrene-treated rats, nuclear DNA binding of BP was increased 300 percent over control values, binding to microsomal protein was increased almost 200 percent, and binding to nuclear protein was decreased almost 50 percent. Further study will be required to determine the implications of this nuclear enzyme for carcinogenesis, cancer prevention, and cancer chemotherapy.

Several alkylating agents enhance the binding of nucleic acids to cellular membranes and, in some cases, to intact living cells (Kubinski et al., 1976). A survey was undertaken to determine how widespread this phenomenon is among other chemical carcinogens. The spectrum of compounds tested and found active included alkylating agents, aromatic amines, hydrocarbons, and carcinogenic metals. The attachment of DNA and RNA to *Escherichia coli* and Ehrlich ascites cells was significantly enhanced in the presence of chemical mutagens and ultimate carcinogens. Precarcinogens and nonultimate carcinogenic metabolites, as well as tumor-promoting agents, did not increase the binding. The only exception to these findings was N-acetoxy-2-acetylaminofluorene, which is thought to be an ultimate carcinogen. While it measurably increased binding of DNA to both cell types, no such effect was observed with RNA. Presumably, binding of nucleic acids in these

experiments was followed by their penetration into the cells, since sensitivity toward nucleases decreased markedly in all cases studied; confirmation by an independent technique will be required to establish this point with certainty.

There is no direct evidence at present that this phenomenon plays any role in the process of tumor induction by these chemicals. However, these compounds generally produce strong cytotoxic reactions, and it is tempting to speculate that, in an organ or tissue exposed to carcinogenic chemicals, nucleic acids may be released from injured cells and then efficiently incorporated into surrounding tissue. Such nucleic acids may carry genetic information (viral or cellular) freed from normal cellular regulatory mechanisms or constraints. Thus, important and perhaps even permanent metabolic alterations may follow incorporation of such modified macromolecules by the recipient cell. DNA exposed to monoalkylating carcinogens and profoundly altered physicochemically, can still transfer genetic information, although significant alterations in genetic linkages are observed (Kubinski and Kubinski, 1976). Enhanced cellular binding of nucleic acids in the presence of carcinogens and mutagens could conceivably form the basis for a mass screening system for suspected chemical carcinogens. Such a system would have the advantages that it is conceptually and technically simple and that the results are known within hours.

Ultraviolet (UV) light is the major cause of human skin cancer. As most types of skin tumors are diagnosable early and can be successfully treated, skin cancer mortality is low. Nevertheless, skin tumors provide a convenient model of tumor development and behavior. Moreover, certain types of chemicals produce cellular damage similar to that of UV light, so that studies of UV-induced skin tumors have a wider application than is generally appreciated.

Patients with the inherited disease xeroderma pigmentosum (XP) are abnormally susceptible to the carcinogenic action of UV light. All such patients thus far studied have defective DNA repair capability although the specific defect is not the same in all XP patients. If the somatic mutation hypothesis of carcinogenesis is correct, the expected frequency of mutations induced by UV light would be higher for cells from XP patients than for normal human cells. The frequency of UV-induced mutations to azaguanine resistance was compared in normal human skin fibroblasts and in fibroblasts from an XP "variant" (Maher et al., 1976). In contrast to cells from classical XP patients, variant cells have a repair defect not involving excision repair of UV-damaged DNA and should, therefore, contain the same number of unexcised lesions as normal cells at any given time. The variant cells in this study nevertheless exhibited a significantly higher-than-normal frequency of induced mutations per dose of UV radiation, providing strong support for the somatic mutation theory of cancer. The variant cells took approximately four times longer than normal cells to restore their DNA molecules to the size found in unirradiated cells. As this size restoration, although delayed, is accomplished long before the next round of DNA synthesis, its relation to increased mutagenesis and carcinogenesis is not immediately apparent.

Studies of UV mutagenesis in cultured cells provide important data relevant to cancer. Direct studies of UV carcinogenesis would be desirable, but attempts to achieve UV transformation of mammalian cells in culture have been almost universally unsuccessful. Malignant transformation of a well-characterized line of mouse fibroblasts has recently been accomplished using UV light plus a tumor promoter, tetradecanoyl phorbol acetate (TPA) (Mondal and Heidelberger, 1976). Transformation was dose-dependent for UV radiation and, in general, transformation frequency decreased if TPA treatment started 96 hours or more after irradiation. These experiments show that UV light acts as an initiator of carcinogenesis in these cells just as it does on mouse skin in living animals. This tissue culture system, therefore, appears to be suitable for investigating the mechanism of ultraviolet carcinogenesis.

Experiments were undertaken to determine the effect of an anticarcinogen, butylated hydroxytoluene (BHT), on DNA damage and repair following administration of the carcinogen 2-acetylaminofluorene (AAF) (Goodman et al., 1976). When rats receiving AAF in their diets were simultaneously fed BHT, the amount of the carcinogen bound to hepatic DNA was reduced. Since BHT did not influence either excision repair or post-replication repair of DNA, it presumably acted by inhibiting the reaction of the carcinogen with DNA rather than by facilitating removal of already bound residues.

In view of the finding that XP can be associated with any one of at least six defects in DNA repair, it was of interest to determine whether cells from patients with this disease have any abnormality in semiconservative DNA synthesis. Rates of semiconservative synthesis were measured in classical XP, XP variant, and normal human skin cells (Rudé and Friedberg, 1977). No differences in rates were detected in unirradiated cells. Exposure to UV radiation caused the rate of DNA synthesis to decrease for at least three hours in all three types of cells. Variant cells were slower than normal cells to recover their normal rate of synthesis, and classical XP cells did not recover their normal rate during a 24-hour observation period following irradiation. When the UV-irradiation to classical XP and XP variant cells was reduced so that survival in all three cell types was similar, recovery of the DNA synthetic rate was also similar in the three. These results indicate that there is no inherent defect in semiconservative DNA synthesis in either classical XP or XP variant cells which is independent of a defect in DNA repair capacity.

Many dietary components, their metabolites, and their contaminants are known to cause cancer in experimental animals. Epidemiologic evidence suggests that some of these substances are also carcinogenic in humans, but except for isolated cases, human cancer has been more frequently associated with specific nutritional deficiencies and/or excesses than with food additives or possible carcinogens contained in specific foods (reviewed in Wynder, 1976). Nutritional deficiencies appear related, for example, to cancers of the stomach, cervix, and thyroid. Overnutrition appears to have a significant role in the development of cancer of the colon, pancreas, kidney, breast, ovary, endometrium, and prostate. Except for cancer of the endometrium and kidney cancer in women, there appears to be no significant relationship to obesity *per se*. Rather, the evidence suggests that

the etiologic factors relate to a high intake of fats and possibly to other variables associated with high fat intake. One hypothesis suggested by these findings is that an alkylating cholesterol derivative and/or cholesterol epoxide might be a ubiquitous initiating carcinogen to the mucosa of the colon, the cortex of the kidney, and ducts of the breast, prostate, and pancreas (Wynder, 1976).

The complexity of natural foods, the known toxicities of some of their components, and the limited testing that natural components of foods have received make it virtually certain that the number of carcinogens discovered in foods will continue to increase (Miller and Miller, 1976). At least two classes of dietary constituents, however, may exert a protective effect against some types of chemical carcinogenesis: (1) inducers of increased microsomal mixed function oxidase activity; and (2) antioxidants, some of which are extensively employed as food additives. In early studies of mixed function oxidases known as aryl hydrocarbon hydroxylase (AHH), it was assumed that high levels of activity are normally present. This assumption is true for the liver, but subsequent studies have shown that most, if not all, AHH activity in the lungs and small intestine is due to dietary inducers (Wattenberg et al., 1976). Indole-3-acetonitrile, indole--3-carbinol, and 3,3'-diindolylmethane were identified as inducers present in Brussels sprouts, cabbage, and cauliflower. Other naturally occurring inducers include flavones, safrole, isosafrole, β-ionone, and oxidized sterols. The antioxidant properties of two widely used food additives, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), appear to be responsible for the ability of these compounds to inhibit the carcinogenic effects of a number of chemicals. Other antioxidants having carcinogen-inhibiting capacities include ethoxyquin, disulfiram, and dimethyldithiocarbamate. The actual mechanism by which antioxidants interfere with carcinogenesis has not been established; it is even possible, since they have other biological activities, that the interference is not due to their antioxidant properties *per se*. Whatever the mechanism, it is clear that dietary modifications can be an important factor in human cancer prevention.

The mechanism by which some antioxidants inhibit chemical carcinogenesis is difficult to determine because the target tissue in most experimental models is distant from the site(s) of administration of carcinogen and antioxidant. Thus, either compound could undergo change before reaching the target organ. To overcome this difficulty, benzo(*a*)pyrene (BP) was administered to mice by oral intubation; this procedure resulted in tumor formation in the forestomachs of more than 90 percent of the treated animals (Borchert and Wattenberg, 1976). If one percent of the antioxidant disulfiram was added to the diet, no tumors occurred at that site. This inhibition of tumor formation was paralleled by inhibition of macromolecular binding of BP to RNA and protein in the forestomach. In contrast to the forestomach, no inhibitory effect on BP binding to DNA, RNA, or protein by disulfiram was found in the liver, which remained free of cancer under the conditions of the experiment.

Rapid cell proliferation, a characteristic neoplasia, is accompanied by membrane synthesis. Since membrane phospholipids contain appreciable amounts of linoleate, the effect of dietary linoleate on the growth rate and on fatty acid composition of mouse mammary tumors was investigated (Rao and Abraham, 1976). Six weeks after tumor transplantation, tumors in recipients fed the linoleate diet weighed 3-4 times more than those in mice fed the fat-free or saturated-fat diets. When the level of linoleate-containing corn oil was increased from 1 to 15 percent, the linoleate content of the tumors increased from 4 to 18 percent of the total fatty acids; the enhanced growth rate and the arachidonate content were similar at both levels. It appears, therefore, that the arachidonate content and the presence of linoleate, rather than its amount, are the factors related to tumor growth in this system.

Safrole (4-allyl-1,2-methylenedioxybenzene) is weakly hepatocarcinogenic when fed to rodents. It is ingested by humans in certain spices and other plant derivatives. The electrophilic reactivities of potential safrole metabolites were studied in vitro (Wislocki et al., 1976). The compound 1'-Hydroxysafrole was slowly metabolized by mouse and rat liver cytosols and microsomes to electrophilic metabolites which are possible ultimate carcinogenic derivatives. Safrole and 1'-hydroxysafrole have not shown mutagenic activity in Salmonella typhimurium assays with or without liver activation systems. Improved activation systems may be required for the reliable detection of the less potent carcinogens via mutagenicity assays.

The glandular stomachs of rodents have been considered altogether refractory to orally administered hydrocarbons. Recently, however, the glandular stomachs of certain strains of inbred Syrian hamsters developed adenocarcinoma after polycyclic hydrocarbon gavage (Homburger et al., 1976). This experiment demonstrates the importance of genetic factors and provides another experimental model for human gastric cancer. This model is especially attractive since spontaneous glandular carcinoma of the stomach is unknown in the Syrian hamster. Moreover, the strains used in this study possess the most susceptible gastric mucosa yet observed in rodents subjected to polycyclic hydrocarbon carcinogenesis. F_1 and backcross experiments may reveal the nature of the genetic control governing susceptibility to gastric cancer. Since ulcerations of the gastric mucosa of the glandular stomach were not observed except in the presence of carcinoma, the observed susceptibility to chemical carcinogens is apparently not related to the gastric ulcer syndrome, as suggested for human gastric cancer. However, these observations raise the possibility that the benzo(a)pyrene in charcoal-broiled steaks may be relevant to human gastric cancer as postulated by Lijinsky and Shubik (1964).

Cancer Biochemistry: The study of tumor and host biochemistry can (a) provide clues about possible mechanisms of action of carcinogens and form a basis for attempts to inhibit or reverse their activity; (b) suggest appropriate biochemical targets for chemotherapy; and (c) reveal abnormalities which can be used to diagnose cancer or to identify persons at high risk of developing cancer.

Prolactin and estrogen are considered the two most important hormones involved in mammary tumorigenesis in female rats, which serve as a model for the human disease. Adrenalectomy in 7,12-dimethylbenz(a)anthracene-treated rats increased the number and size of mammary tumors and the level of serum prolactin, as compared with intact controls (Chen et al., 1976). The increased tumor growth depended upon increased prolactin release. The effect of adrenalectomy in these rats is the opposite to the effect reported in women with breast cancer. However, adrenalectomy for human breast cancer is usually accompanied by ovariectomy, whereas in this study the rats' ovaries were not removed. The effect of adrenalectomy alone on prolactin release in women is not yet known.

Ovariectomy causes a slight decrease in prolactin receptors in 7,12-dimethylbenz(a)anthracene-induced rat tumors regardless of whether the tumors regress or continue to grow (Costlow et al., 1976). The affinity of the receptor for prolactin remains unchanged. Autonomous and ovariectomy-responsive mammary tumors in this system can not therefore be distinguished on the basis of prolactin receptor sites. Hypophysectomy, which causes a prompt 10-fold decrease in prolactin receptors in rat liver, causes only a slight reduction in the receptors in mammary tumors from these same animals. Endocrine regulation of prolactin receptors is thus distinctly different in normal liver and neoplastic mammary tissue. Since autonomous tumor growth is not necessarily associated with a loss of tumor prolactin receptors, the failure of prolactin regulation of tumor growth may reflect a deficiency in the prolactin response system beyond the initial binding step.

Epidemiologic data suggest an association of changed hormone metabolism with the development of breast cancer in humans. A recent study (Hill et al., 1976) compared the plasma androgen, estrogen, and gonadotropin levels in young premenopausal Bantu women at low risk for breast cancer and in Caucasian women at high-risk. Plasma estrodiol was significantly lower than normal in Bantu, but not in Caucasian, women with breast cancer, while the ratio of plasma androstenedione to dehydroepidandrosterone increased in Bantu patients. These changes in the Bantu women imply a change in ovarian and/or adrenal activity. Lack of similar hormonal changes in Caucasian patients may reflect initial differences in the hormone profile between healthy Caucasian and Bantu women and different environmental factors that promote breast cancer in Caucasian women. Adrenal activity may be subject to dietary control, and the Bantu diet, which is low in fat and animal protein, may maintain a hormone profile that renders the Bantu women less susceptible to the development of breast disease. Further studies of different populations will be necessary to determine the precise relationship of diet to hormone profile and breast cancer.

Many anticancer drugs affect enzymes in crucial metabolic pathways. Since the specificity of these drugs depends on enzyme differences in normal and malignant cells, it is imperative to identify consistent enzyme differences which can be exploited therapeutically. Despite claims that there are no biochemical differences between normal and cancer cells or that, if such differences exist, they are merely quantitative, the evidence for enzymatic imbalance in cancer cells is now extensive. The evidence can be integrated

into a pattern linked with either malignant transformation or tumor progression or both (Weber, 1977). Enzyme imbalance is characteristic of malignancy. Other rapidly dividing tissues such as fetal, developing, and regenerating liver have different enzymic profiles although they have similar growth rates. Studies of enzymic profiles in a series of transplantable, chemically induced rat hepatomas with different growth rates led to the "molecular correlation concept" (Weber, 1977), which holds that the enzymic pattern of cancer cells has both ordered and random components. The findings in the rat hepatomas have since been extended to other tumors, including "spontaneous" human tumors and chemically and virally induced tumors in other species.

According to the molecular correlation concept, the ordered pattern is manifested in the key enzymes, the ones that are stringently linked with neoplastic transformation and progression. The enzymatic alterations present in all neoplasms with the same tumor cell type are transformation linked; such enzymes are markers of neoplasia and should be useful in diagnosis. The activities of several key enzymes and the pattern of enzymatic and metabolic imbalance change with the degree of malignancy exhibited by the tumors; these are progression-linked alterations. The covariance of the activities of key enzymes can characterize tumor malignancy and growth rate. In contrast, enzymes present in excess are not key enzymes and their activities fluctuate randomly. Since the activity and isozyme pattern of key enzymes are linked with neoplastic transformation or progression, the key enzymes should be the most sensitive targets for the rational design of selective chemotherapy.

Nuclear magnetic resonance (NMR) measurements on tissue samples have shown that water proton relaxation times differ among tissues and organs, and are often altered in disease states including cancer. If such measurements were feasible on live animals and could be used to generate images in which altered relaxation times were correlated with specific anatomic structures, zeugmatography, a technique based on NMR measurements, could become important in cancer diagnosis. Live mice with implanted mammary tumors were studied at 8.13 MHz (Lauterbur et al., 1976). Field scans produced a locally saturated signal whose amplitude depended on the average local spin-lattice relaxation time T_1 as well as on the amount of water present; the scans were used to generate T_1-dependent images by reconstruction. The experiment was designed to give only low resolution images. Faster, higher resolution techniques are being refined, and equipment large enough to permit NMR zeugmatographic imaging on humans is being developed. NMR zeugmatography has the advantages that bones and air do not interfere with it and that it does not involve ionizing radiation.

Projections

The programmatic emphasis is on Environmental Carcinogenesis with particular reference to studies on: (a) the mechanisms of action of chemical and physical cancer causing agents, (b) dietary, hormonal, and other means of inhibiting the response of the host to chemical and physical carcinogens, (c) the further assessment of the role of promoters in environmental carcinogenesis, as well as studies on the modification of cellular response to

promoters, (d) the development of tests for the preclinical diagnosis of cancer, and (e) the development of rapid, relatively inexpensive tests for the evaluation of the carcinogenicity of environmental substances. The development of knowledge concerning the stepwise processes by which a cell becomes cancerous is of fundamental importance relative to the pursuit of these and related cancer research objectives.

Other Activities

1. Workshop on Tumor Promotion and Cofactors in Carcinogenesis, held at Woods Hole, Massachusetts, October 12-14, 1976 and sponsored by the Division of Cancer Research Resources and Centers.

2. Workshop on DNA Repair and Carcinogenesis, held at Alexandria, Virginia, December 8-10, 1976 and jointly sponsored by the Division of Cancer Research Resources and Centers and the Division of Cancer Cause and Prevention.

CARCINOGENESIS PROGRAM

SUMMARY BY SUB-CATEGORY
(Dollars in Thousands)

	NONCOMPETING		COMPETING		TOTAL	
	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT
CHEMICAL CARCINOGENESIS	111	$6,442	66	$4,299	177	$10,741
BIOCHEMISTRY OF CANCER AND CANCEROUS HOSTS	64	3,966	18	1,621	82	5,587
BASIC PROGRAM PROJECTS	5	5,328	1	342	6	5,670
TOTAL CARCINOGENESIS	180	$15,736	85	$6,262	265	$21,998

REFERENCES

Argus, M. F. and Arcos, J. C., Hydrocarbon-nitrosamine Synergism as a Possible Amplifying Factor in Lung Tumorigenesis by Tobacco Smoke. J. Theor. Biol. 56:491-498, 1976. (CA 13206)

Argus, M. F. and Hoch-Ligeti, C., Comparative Study of the Carcinogenic Activity of Nitrosamines. J. Nat. Cancer Inst. 27:695-709, 1961. (CA 04939)

Borchert, P. and Wattenberg, L. W., Inhibition of Macromolecular Binding of Benzo(a)pyrene and Inhibition of Neoplasia by Disulfiram in the Mouse Forestomach. J. Nat. Cancer Inst. 57:173-179, 1976. (CA 15638, CA 14146)

Chen, H.-J. J., Bradley, C. J., and Meites, J., Stimulation of Carcinogen-Induced Mammary Tumor Growth in Rats by Adrenalectomy. Cancer Res. 36:1414-1417, 1976. (CA 10771)

Costlow, M. E., Buschow, R. A., and McGuire, W. L., Prolactin Receptors in 7,12-Dimethylbenz(a)anthracene-Induced Mammary Tumors Following Endocrine Ablation. Cancer Res. 36:3941-3943, 1976. (CA 11378, F22-04004)

Goodman, J. I., Trosko, J. E., and Yager, J. D., Jr., Studies on the Mechanism of Inhibition of 2-Acetylamino-fluorene Toxicity by Butylated Hydroxytoluene. Chemico Biol. Interact. 12:171-182, 1976. (CA 13344, CA 13048, K04-CA-27085, CA 07175)

Hammond, E. C., Selikoff, I. J., and Lawther, P. J., In First Fall Scientific Assembly of the American College of Chest Physicians, Chicago, October 30, 1969.

Hill, P., Wynder, E. L., Helman, P., Hickman, R., and Rona, G., Plasma Hormone Profiles in Populations at Different Risk for Breast Cancer. Cancer Res. 36:1883-1885, 1976. (CA 12376)

Homburger, F., Handler, A. H., Soto, E., Hsueh, S. S., Van Dongen, C. G., and Russfield, A. B., Adenocarcinoma of the Glandular Stomach Following 3-Methylcholantrene, N-Nitrosodiethylamine, or N-Nitrosodimethylamine Feeding in Carcinogen-Susceptible Inbred Syrian Hamsters. J. Nat. Cancer Inst. 57:141-144, 1976. (CA 15425, CA 13232)

Kubinski, H. and Kubinski, Z. O., Effects of Monoalkylating Carcinogens and Mutagens on Transforming DNA. Fed. Proc. 35:1660, 1976. (CA 16989, CA 08059)

Kubinski, H., Morin, N. R., and Zeldin, P. E., Increased Attachment of Nucleic Acids to Eukaryotic and Prokaryotic Cells Induced by Chemical and Physical Carcinogens and Mutagens. Cancer Res. 36:3025-3033, 1976. (CA 16989, CA 08059)

Lauterbur, P. C., Lai, C.-M., Frank, J. A., and Dulcey, C. S., Jr., In Vivo Zeugmatographic Imaging of Tumors, 4th Int. Conf. on Med. Physics, Ottawa, July 25-30, 1976. (CA 15300)

Lijinski, W. and Shubik, P., Benzo(a)pyrene and Other Polynuclear Hydrocarbons in Charcoal-Broiled Meat. Science 145:53-55, 1964.

Maher, V. M., Ouellette, L. M., Curren, R. D., and McCormick, J. J., Frequency of Ultraviolet Light-induced Mutations is Higher in Xeroderma Pigmentosum Variant Cells Than in Normal Human Cells. Nature 261:593-595, 1976. (CA 13058)

Marquardt, H., Grover, P. L., and Sims, P., In Vitro Malignant Transformation of Mouse Fibroblasts by Non-K-Region Dihydrodiols Derived from 7-Methylbenz(a)anthracene, 7,12-Dimethylbenz(a)anthracene, and Benzo(a)pyrene. Cancer Res. 36:2059-2064, 1976. (CA 08748, CA 15205, K4-CA 00127)

Miller, J. A. and Miller E. C., Carcinogens Occurring Naturally in Foods. Fed. Proc. 35:1316-1321, 1976. (CA 07175, CA 15785, T 01 05002)

Mondal, S. and Heidelberger, C., Transformation of C3H/10T1/2 CL8 Mouse Embryo Fibroblasts by Ultraviolet Irradiation and a Phorbol Ester. Nature 260:710-711, 1976. (CA 07175)

Rao, G. A. and Abraham, S., Enhanced Growth Rate of Transplanted Mammary Adenocarcinoma Induced in CSH Mice by Dietary Linoleate. J. Nat. Cancer Inst. 56:431-432, 1976. (CA 11736)

Rudé, J. M. and Friedberg, E. C., Semi-Conservative Deoxyribonucleic Acid Synthesis in Unirradiated and Ultraviolet-irradiated Xeroderma Pigmentosum and Normal Human Skin Fibroblasts. Mutation Res. 42:433-442, 1977. (CA 12428, K04 CA 71005)

Van Duuren, B. L. and Goldschmidt, B. M., Cocarcinogenic and Tumor Promoting Agents in Tobacco Carcinogenesis. J. Nat. Cancer Inst. 56:1237-1242, 1976. (CA 13343)

Vaught, J. and Bresnick, E., Binding of Polycyclic Hydrocarbons to Nuclear Components In Vitro. Biochem. Biophys. Res. Comm. 69:587-591, 1976. (CA 17565)

Vaught, J. B., Howard, E. F., and Bresnick, E., Breakage of Human Cell DNA After Exposure to 3-Methylcholanthrene-11,12-oxide. Cancer Res. 36:272-275, 1976. (CA 12906)

Wattenberg, L. W., Loub, W. D., Lam, L. K., and Speier, J. L., Dietary Constituents Altering the Responses to Chemical Carcinogens. Fed. Proc. 35:1327-1331, 1976. (CA 09599, CA 14146)

Weber, G., Enzymology of Cancer Cells. New Eng. J. Med. 296:486-493, 541-551, 1977. (CA 05034, CA 13526)

Weinstein, I. B., Jeffrey, A. M., Jennette, K. W., Blobstein, S. H., Harvey, R. G., Harris, C., Autrup, H., Kasai, H., and Nakanishi, K., Benzo(a)pyrene Diol Epoxides as Intermediates in Nucleic Acid Binding In Vitro and In Vivo. Science 193:592-595, 1976. (CA 02332, CA 11572)

Wislocki, P. G., Borchert, P., Miller, J. A., and Miller, E. C., The Metabolic Activation of the Carcinogen 1'-Hydroxy-safrole In Vivo and In Vitro and the Electrophilic Reactivities of Possible Ultimate Carcinogens. Cancer Res. 36:1686-1695, 1976. (CA 07175, CA 15785, T 01 05002)

Wynder, E. L., Nutrition and Cancer. Fed. Proc. 35:1309-1315, 1976. (CA 12376, CA 16382)

CANCER EPIDEMIOLOGY PROGRAM

Description

Epidemiology, biometry, and human population genetics offer research approaches applicable to a broad range of problems in cancer biology, cause, prevention, diagnosis, and treatment. Biometric research is so much a part of substantive research that it is often reported without particular recognition of the statistical or mathematical contribution. The Epidemiology Program provides for contact with other disciplines and accommodates research in which the major focus is biomathematics, biometrics, or systems engineering.

Epidemiologic and human population genetics research is primarily oriented toward cancer prevention. Research identifying risk factors, assessing the strength of their association with cancer, and evaluating the potential effects of modifying the human exposure to these risks is a major component of the Epidemiology Program. The smaller biometric component of the program contributes heavily to research citing programmatic support. Epidemiologic projects are usually designed to answer specific questions, are funded for the duration of data gathering and analysis, and then terminate. Consequently the program is characterized primarily by short-term grants (three-four years) and rapid turnover.

In 1977, grantees of the program published on epidemiologic investigations concerning breast cancer, lymphoid malignancies and leukemia, uterine-cervical cancer, and head and neck cancers, as well as investigations relating to specific etiologic factors such as viral agents, X-ray, chemicals, endogenous microflora and clonal origins of various human malignancies.

Biometric investigations included a series of applications of the Mantel-Haenszel procedure, procedures for dealing with censored data in clinical trials, methods of assessing survivorship, and procedures for dealing with special problems in pedigree analysis and disease clustering.

Accomplishments

A study of 156 patients with nasopharyngeal squamous cell cancer and 267 controls derived from the Los Angeles Cancer Surveillance Program between 1971-1974 reported a relative risk of 1.8 associated with history of prior ear, nose, or throat disease, and a relative risk of 2.0 associated with prior occupational exposure to fumes, chemicals, and dusts (Henderson, 1976). Among Chinese and other Asians and Mexican-Americans, a 2.1 relative risk was associated with foreign birthplace. A genetic variation in susceptibility is suggested by increased risk among foreign-born, occupationally exposed Chinese patients exhibiting HLA-A2 and less than two antigens at the B locus.

Drinking, smoking, and dietary patterns of a group of head and neck cancer patients were compared with the same patterns for a matched sample of

alcoholic individuals and a control sample of patients with cancers of sites thought not to be smoking-related. For the sample matched by tobacco use, the head and neck cancer patients were intermediate between alcoholics and patients with cancers of other sites in the quantity of alcohol consumed. When alcoholic individuals were matched with head and neck cancer patients with respect to alcohol use, the head and neck patients were found to use significantly more tobacco than alcoholics while patients with cancers of other sites smoked significantly less than either group. When head and neck cancer patients were matched with alcoholics for tobacco and alcohol use, no differences in dietary patterns were found, but the investigators point out that this could result from the insensitivity of their diet survey methods. When alcoholics and head and neck cancer patients were matched for quantity of alcohol consumption and smoking, the head and neck cancer patients were found to be three times more likely to consume distilled spirits than were alcoholics, and the head and neck cancer patients were almost three times more likely to be daily drinkers than were alcoholics (Feldman, 1976).

A study of 28 primary hepatic carcinoma cases and their close relatives compared with 28 controls and their relatives was carried out in Dakar, Senegal (Larouze, 1976). Assays of sera for hepatitis B surface antigens, antibody to hepatitis surface antigen, and antibody to hepatitis core antigen revealed large differences between the cases and their relatives on the one hand and the controls and their relatives on the other. Both groups were living in areas endemic for hepatitis exposure. Even though small numbers are involved, the findings are consistent with a hypothesis that primary hepatic carcinoma cases were infected with Herpes B Virus by their mothers and that an environmental factor influenced the immune responses of all family members as compared with controls. Further investigations are planned to increase the numbers and to explore possible modes of hepatitis infection as well as immune responses.

An increased risk of breast cancer is experienced by women who have early menarche with delayed first pregnancy and menopause. The risk may be related to different concentrations of various estrogen fractions which women experience from early life. The urine estriol ratio, the concentration of estriol relative to the sum of the concentrations of estrone and estradiol, differs among populations of women with varying susceptibility to breast cancer. It is uncertain whether differences in the estrogen ratio may cause relative infertility and increased risk of breast cancer, or if early pregnancy contributes to a physiologic alteration of the estrogen ratio. Cole et al, studying the urine estriol ratio in women who had recently delivered their first children found that the women aged 19-23 had a follicular phase estrogen ratio 40 percent higher than older primipara with a significant difference in the estrogen ratio during the luteal phase as well. The estrogen ratios of parous women were higher than the levels for nulliparous. The results are not explained by differing frequencies of ovulation or by confounding from breast cancer risk indicators.

Henderson (1977) also investigating breast cancer etiology, summarized a series of studies relating to hormones and breast cancer. Familial investigations

of women with breast cancer suggest an underlying endocrinopathy which can be demonstrated among first-degree relatives. A study of first-degree relatives of women with bilateral breast cancer, women at unusually high risk for breast cancer, is under way.

Patients with certain benign breast diseases are at excess risk of breast cancer. Some investigators believe that this excess risk indicates occult cancer present at the time the benign breast disease was diagnosed. Monson (1976) reports an excess risk of dying from breast cancer which persists as long as 30 years after the diagnosis of chronic cystic mastitis. This persistent risk is unlikely to result from an occult carcinoma confused with cystic mastitis.

Two studies concerned with clustering of acute lymphatic leukemia in children suggested diverse etiologic factors. Vianna and Polan (1976) report on prenatal exposure to varicella as a possible risk. Feldman et al (1976) report acute lymphatic leukemia over a 15-year period in six young females in a genetically isolated Syrian-Jewish community in Brooklyn, New York. The findings could be explained by a maternally transmitted factor. However, the cases were related, and a genetic factor was postulated which may involve the father's age.

A study has shown a seven-fold risk of nodular sclerosing Hodgkin's disease among siblings of similar age and sex, implying exposure to a common factor during childhood, or possibly the transmission of an agent from one to the other many years prior to the onset of frank disease (Grufferman, 1977).

Smith and Pike (1976) have published a procedure for testing the significance of household aggregations of disease in situations where different strata are at varying risk. This procedure is superior to methods that assume all individuals equally susceptible. The improved procedure involves modifications of Mathen and Chakraborty's Z and of Walter's N and suggests a further test statistic T.

Mantel has published several papers dealing with appropriate and inappropriate applications of the Mantel-Haenszel analysis, including a modification for recovery of interlitter information, applied to testing of tumorigenic drugs in animal litters.

A method for solving two related problems in pedigree analysis has been reported. The first application involves fitting familial modes of transmission of a trait to pedigree data and includes chromosome linkage studies as a special case. The second application uses models to calculate probability that a person will be affected with a disease. Genetic, environmental, and combined models can be considered, and pedigrees of any complexity, including consanguinity, can be handled (Skolnick, 1976).

Emphasis and Projections

Areas of current and future emphasis include genetic and environmental interaction in cancer risk, immune deficiencies in cancer susceptibility,

environmental or shared exposures in neoplasia which display clustering or strong environmental associations, and refinement of epidemiologic, population, genetic, and biometric research methods. Two special resources exist in which genealogic background and cancer experience for defined populations are known. Utilization of these resources for research on relationships between genetic and environmental risks await the development and application of new biometric methods for data analysis, as well as the evolution of epidemiologic approaches for studying the absence of disease as a positive factor related to cancer prevention. Furthermore, certain neoplasia which ordinarily occur rather infrequently such as nodular sclerosing Hodgkin's disease, leukemia, multiple myeloma, rhabdomyosarcoma, and melanoma have recently shown evidence of an increased risk associated with shared environmental exposure and/or interesting clustering of cases. Until now, risk factors for multiple myeloma have been unexplored. Better techniques for exploring environmental risks are needed. For example, the lag between environmental exposure and diagnosis of malignancy has frustrated efforts to correlate exposures such as food or toxic substances with cancer risk for sites such as the colon. Methods to validate, recall, or quantify prior exposure are needed and will be explored in the future.

Other Program Activities

In August 1976, a workshop convened to consider contributions which could be made by studying groups at low risk of cancer. Two types of studies were discussed--epidemiologic studies of low-risk groups such as Seventh-Day Adventists, Mormons, Spanish-Americans, Indians, and Orientals, and genealogic studies of Mormons, Spanish-Americans, and Icelandics. The review of work in progress led to the conclusion that two follow-up workshops should be held, with specific work to be accomplished in the interim and with resource publications to derive from the follow-up. One such workshop will be concerned with comparable data and special methods required for studying low-risk groups. The chairman and editor-in-chief for the monograph is Dr. Brian MacMahon. An interim planning session has resulted in the identification of data sets to be prepared, the definition of disease coding to be used, tentative list of participants, date, and site. The other workshop is concerned with clarification of analytic methods for distinguishing environmental from genetic risks for cancer and with special methods for handling large data sets involving geneology and disease experience. This workshop is planned by Dr. Jack Schull and Mr. William Haenszel, and is to result in a publication updating Dr. Schull's 1963 book on genetics and epidemiology. The methods and model data sets to be used, tentative list of participants, date, and site have been planned. Both of these workshops are expected to take place during mid to late fiscal year 1978.

The Program also sponsored the 1976 meeting of epidemiologists, statisticians, and data coordinators associated with cancer centers. In conjunction with this, there were a number of planning workshops--one concerned with definitions to accompany the Centralized Cancer Patient Data Set and another concerned with alternatives to death as measures of survivorship.

Also during fiscal year 1977, three activities were undertaken in collaboration with the Society for Epidemiologic Research (SER). First, a statement clarifying the role of epidemiologic studies vis-a-vis access to patient medical records was drafted for submission to the Privacy Study Commission. A second major interest of the SER was related to training, and this has led to conferences with National Institutes of Health (NIH) staff and to collaboration in developing a study of manpower needs to assist NIH and the National Research Council Research Manpower Study Committee. The third activity concerns the stimulation of young investigators' interest in cancer research. A special workshop to expose young investigators to outstanding cancer epidemiologists is scheduled for June 1977.

A great deal of time and attention has been devoted to the establishing of a Centralized Cancer Patient Data System (CCPDS), to be serviced by a Statistical Analysis and Quality Control Center (SAQC). This data system is to register patients from 19 participating cancer centers. The responsibility for supervising the contract for the SAQC center and the CCPDS grants to comprehensive cancer centers has been delegated to the Epidemiology Program. Recommendations for developing this data system consistent with the development of epidemiologic/statistical/data management capabilities in cancer centers have been formulated.

Interaction with other Epidemiology and Biometry activities of the National Cancer Program (NCP) has continued through attendance of contract review and other working committees, including the Breast Cancer Task Force Epidemiology Committee and the Biometry and Epidemiology Committee.

A new development, the formation of an NIH Epidemiology Committee to help resolve problems of a broader nature, is very promising.

EPIDEMIOLOGY PROGRAM

(Dollars in Thousands)

NONCOMPETING		COMPETING		TOTAL	
NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT
39	$7,403	19	$2,225	58	$9,628

REFERENCES

Cole, P., Brown, J., and MacMahon, B., Oestrogen Profiles of Parous and Nulliparous Women. The Lancet 596-598, 1976. (CA 06373)

Feldman, J., Relationship of Smoking and Drinking to Risk of Head and Neck Cancer. Presented to the 104th Annual Meeting of the American Public Health Association, Epidemiology Section, Miami Beach, Florida, October, 1976. (CA 18073)

Feldman, J., Lee, S., and Seligman, B., Occurrence of Acute Leukemia in Females in a Genetically Isolated Population. Cancer 38:2548-2550, 1976. (CA 18073)

Grufferman, S., Cole, P., Smith, O., and Lukes, R., Hodgkin's Disease in Siblings. N. Engl. J. Med. 296:248-250, 1977. (CA 22104)

Henderson, B., Gerkins, V., Pkie, M., and Casagrande, J., Endocrine Function and Breast Cancer. Genetics of Human Cancer. Edited by J. Mulvihill, R. Miller, and J. Fraumeni, Raven Press, New York, 1977. (CA 17054)

Henderson, B., Louie, E., Jing, J., Brull, P., and Gardner, M., Risk Factors Associated with Nasopharyngeal Carcinoma. N. Engl. J. Med. 29:20, 1101-1106, 1976. (CA 17054)

Larouze, B., Saimot, G., Lustbader, E., London, W., Werner, B., Payet, M., and Blumberg, B., Host Responses to Hepatitis Infection in Patients with Primary Hepatic Carcinoma and Their Families: A Case-Control Study in Senegal, West Africa. The Lancet 534-538, 1976. (CA 06551)

Mantel, N. and Mantel-Haenszel, Analysis of Litter Matched Time to Response Data with Modifications for Recovery of Inter Litter Information. A Manuscript. 1976. (CA 16586)

Monson, R., Yen, S., MacMahon, B., and Warren, S., Chronic Mastitis and Carcinoma of the Breast. The Lancet 224-226, 1976. (CA 06373)

Skolnick, M., de Nevees, K., Canning, C., and Sredharan, R., Computer and Biomedical Research 9:393-407, 1976. (CA 16573)

Smith, P. and Pkie, M., Generalizations of Two Tests for the Detection of Household Aggregation of Disease. Biometrics 32:817-828, 1976. (CA 17054)

Vianna, N. and Polan, A., Childhood Lymphatic Leukemia: Prenatal Seasonality and Possible Association with Congenital Varicella. American Journal of Epidemiology 103:3, 321-332, 1976. (CA 12707)

VIRAL ONCOLOGY PROGRAM

Description

Viral Oncology is concerned with the role of viruses in the etiology of cancer and the consequences of this for the identification of susceptible individuals, early diagnosis, and development of new modes of prevention and treatment. Investigations comprise studies at the cellular and molecular levels, as well as the level of the host, in model systems and in man. They include the nature and mechanism of neoplastic transformation and its expression at all levels of biological organization, the biology and biochemistry of the cell-virus and host-virus relationship, pathogenesis, pathology and immunology of virus-induced neoplastic diseases, and basic research relevant to cancer, such as investigations of gene expression and cell regulatory functions, in which viruses are important tools.

The Program emphasizes the nature and mechanisms of virus-induced neoplastic transformation and alterations in the cell that characterize it as neoplastic. These are key questions about cancer which virus research has the most promise to elucidate; understanding of these problems will contribute significantly to the control of cancer. This emphasis on the mechanism of transformation and its fundamental expression in a wide array of model systems serves to distinguish the grant-supported from the complementary contract-supported program which focuses on the role of viruses in human cancer.

The main goals of the Viral Oncology Grants Program are to identify viral genes and their products responsible for neoplastic transformation and for the maintenance of the transformed state. Research to learn how these genes or their products interact with cellular genes and which cellular genes are so involved is encouraged. Learning how these interactions impinge upon the appearance of new cellular functions is a further objective of this program. Such studies are vital to understanding neoplastic transformation, which, of course, results in new cellular functions. Insights of this sort may also suggest strategies to counteract abnormal functions and reprogram malignant cells to become responsive again to normal growth controls.

No approach today can compete with the power of viruses to induce oncogenic transformation at will in vitro. The analysis of this signal event and of the resulting neoplastic diseases has resulted in a vast number of concepts, reagents, and techniques, whose impact on human cancer is still developing. Furthermore, the interaction of viruses and cells provides specific markers which permit following the intricacies of these reactions in cells. From this viewpoint, tumor viruses may be looked upon as basic tools to study how and why some genes are expressed and others are repressed, and thus how cell functions are controlled during normal as well as abnormal growth and differentiation. These considerations place the study of tumor viruses in the forefront of modern biology as one of the important branches of cell science.

Recent Accomplishments Related to the Cause and Prevention of Cancer

Impressive advances have been made in delineating the physical and functional elements of tumor virus genomes, i.e., determining the sequence of nucleotides in the genome responsible for a particular function and locating that sequence in the genome. Thus, the genome of nondefective avian tumor viruses consists of 10,000 nucleotides, particular sequences of which code for at least four functions: reverse transcriptase ("pol"), internal viral proteins ("gag"), envelope glycoprotein ("env"), and transformation ("src"). Additional evidence indicates that the responsible sequences are linked together in the following order starting from the 5' ("capped") end of the genome: gag-pol-env-src. Viral mutants that lack one or another of these functions and advances in chemical and physical analyses of genes have been invaluable in these investigations. Such studies sort out the interaction of viral genes with cellular genes and elucidate the manifold consequences of these interactions (L. Wang, 1976).

According to one group of investigators, the avian virus gene for transformation (src) may have evolved from a normal sequence of DNA nucleotides of chickens. There is striking, though imperfect, homology between "src" and these normal sequences. The fact that there is no analogous cellular sequence for "env," the viral gene essential for replication, emphasizes the uniqueness of src in its relationship to the genes of normal cells (Stehlin et al., 1976). The normal function of this sequence is unknown, but the possibility that it is concerned with cellular differentiation is under consideration in many laboratories.

Another example of the analytical approach concerns the avian virus gene essential for virus replication, i.e. "env." The genome of the Bryan mouse sarcoma virus is defective, lacking the "env" gene and therefore is deficient in virus production. It has, however, the "src" gene and is able to transform cells. The genome of RAV-2 virus is similar to the Bryan strain but lacks "src," so while capable of virus production, it is nontransforming. Stacy et al., 1977 micro-injected RAV-2 RNA or various messenger RNA's (mRNA's) derived from RAV-2 infected cells into cells transformed by the Bryan virus. A 21S mRNA had the greatest activity in converting the Bryan virus to an infectious virus, i.e., providing it with the "env" element it previously lacked. This indicates that the 21S mRNA is the messenger for "env." Curiously, the RAV-2 viral RNA had little of this particular messenger activity although it had messenger activity for other viral functions. This point remains unexplained.

The in vitro synthesis of the infectious DNA of an RNA tumor virus was recently accomplished (Rothenberg et al., 1977). Production of proviral infectious DNA is a necessary event in oncogenic transformation by these viruses. Preparing pure proviral DNA and investigating the details of its synthesis will be simplified by isolating the system from the complex milieu of the cell. This achievement is another instance of the in vitro synthesis of a functional gene mediated by that powerful reagent, the reverse transcriptase of tumor viruses.

Little is known about the site of viral or proviral DNA integration, a key point in transformation. Two reports address themselves to this question.

In one (N. Battula and H. M. Temin, 1977), it appears that spleen necrosis virus (a virus closely related to the RNA tumor viruses and a convenient tool to study this question) integrates at a single site in chronically infected cells but at multiple sites in acutely infected cells. Apparently survival of the infected cell (and presumably of transformed cells in the case of a transforming virus) is dependent on whether integration is single or multiple. On the other hand, with SV 40, integration, though it may take place in one or two particular chromosomes (C. Croce, 1977), is so generalized within those chromosomes that a single integration site is precluded (M. Botchan, et al., 1976). While integration may be nonspecific in this case, it may, as a matter of chance, also occur at some special site which results in transformation. Similar results with SV 40 were obtained by G. Ketner and T. J. Kelly, Jr., 1976.

A variety of factors determines whether a virus can infect and replicate in a cell. In the mouse leukemia ecotropic viruses, the so-called Fv-1 gene of the mouse is one of those controlling elements. When this gene is dominant, virus replication is inhibited. Now, two groups of molecular virologists agree on the underlying cause of this host restriction. While proviral (infectious) DNA is equally well produced by the virus in permissive (i.e., permitting viral replication) and nonpermissive cells, only in permissive cells does the proviral DNA integrate with cellular DNA. The Fv-1 gene thus controls integration of proviral DNA. Since integration is also essential for transformation, an understanding of this control is important in the continual quest to analyze oncogenic transformation (P. Jolicoeur and D. Baltimore, 1976; M. M. Sveda and R. Soeiro, 1976).

Endogenous RNA tumor viruses occur naturally in a host. "Ecotropic" endogenous viruses are considered infectious and oncogenic in their natural host, whereas "xenotropic" viruses are not. The role of xenotropic viruses in their natural host is presently obscure. Some new data, however, suggest that the xenotropic virus of the AKR mouse acts synergistically (possibly by recombination) with its ecotropic virus to induce naturally occurring leukemia in this high leukemia mouse strain. The level of xenotropic virus increases in the thymus with age, whereas the level of the ecotropic virus remains unchanged (K. Kawashima, et al., 1976). Since this disease originates in the thymus and its incidence increases with age, the idea that it results from an interaction of these viruses is plausible.

Bat cells infected with the bovine leukemia virus continuously release large quantities of virus (H. C. McDonald and J. F. Ferrer, 1976). Exogenous infection of normal cells with this virus, a prerequisite to a fundamental investigation of the process, has been a difficulty in the past. The availability of sufficient virus will accelerate research in leukemia to determine the significance of bovine leukemia to public health.

An intriguing discovery is the fact that human serum has an extraordinary lytic activity for a wide variety of oncornaviruses compared to the sera of other species (R. M. Welsh, Jr., 1976). Furthermore, antibody does not appear to be involved. The difficulties of demonstrating a significant association between C-type viruses and human neoplasias may be a consequence

of this phenomenon. This observation suggests a further mechanism in man to protect him against C-type (oncorna-) viruses.

A related observation indicates that normal mouse serum contains a factor that specifically neutralizes xenotropic oncorna viruses (J. C. Leong, et al., 1977). The factor is associated with a serum lipoprotein and does not involve antibody. Such a factor interacting with viral antigens on the cell surface could affect cellular functions and thus play a role in regulating normal life processes--for example, differentiation. It may also explain why xenotropic viruses are non-oncogenic in their natural host.

The possibility of developing a simple diagnostic blood test for cancer is suggested by recent results with the mammary cancer of mice. A very sensitive test for a glycoprotein component of mouse mammary cancer, gP52, was devised. The level of gP52 in the plasma of mice parallels the presence and extent of mouse mammary cancer (E. Ritzi, et al., 1976).

Substantiating evidence has been adduced to implicate viruses in human cancer (Prochownik and Kirsten, 1977). The spectre of adventitious contaminants haunts all claims to the isolation of tumor viruses from human tissue. In this instance, DNA sequences related to simian sarcoma virus were demonstrated in the cells of fresh autopsy tissue of patients with malignancies, arguing against contamination as an explanation for the results.

Recent Accomplishments Related to the Biology of Cancer

Bits of autologous or heterologous spleen implanted subcutaneously into splenectomized Friend leukemic mice prolonged their lives and in some instances cured them. These unexpected results demonstrate that the malignant process can be altered at a relatively late stage in the disease and suggest a novel approach to reversing an established malignancy (S. B. Levy, 1976).

Charlotte Friend and co-workers (1971) opened the field of erythropoiesis *in vitro* by discovering that dimethyl sulfoxide (DMSO) induced differentiation of cultured Friend hemoblasts. Recently powerful new inducers of erythroid differentiation have been discovered. One class of inducers, consisting of purine and purine analogues, includes the purine hypoxanthine, which is almost one hundred times more potent than DMSO (J. F. Gusella and D. Housman, 1976). The induction of differentiation by naturally occurring substances may reflect a mechanism in normal hemopoiesis.

A cell surface protein termed LETS (Large External Transformation Sensitive) is reduced, in some instances to the extent that it is undetectable, in cells transformed *in vitro* by adenoviruses. Recent results show a more or less quantitative correlation between the ability of such transformed cells to produce invasive cancer and the loss of LETS, thus further emphasizing the significance of this protein in tumorigenesis. The manner in which loss of LETS confers oncogenic properties to the transformed cell is still obscure.

The technique of introducing drugs and metabolites into cultured cells, using lipid vesicles as carriers, suggests that by manipulating the lipid composition

of vesicles it may be possible to achieve some degree of "targeting" to specific regions of the cell. Vesicles that enter cells by endocytosis would be directed into lysosomal apparatus, whereas, fusion with the plasma membrane would release the vesicle contents directly into the cytoplasm and could also provide a method for modifying the composition of the plasma membrane (G. Poste and D. Papahadjopoulos, 1976). The recent development of large unilamellar vesicles has made possible the incorporation of proteins, nucleic acids, and in general, substances too large to be entrapped in conventional classes of vesicles (D. Papahadjopoulos, et al., 1975). The ability to introduce a variety of natural and synthetic substances into particular cells and in particular parts of a cell will provide a valuable technique for basic investigations of the cell and possibly for chemotherapy.

A method of gene purification and amplification, which can be applied to any structural gene whose messenger RNA (mRNA) can be obtained, was recently developed. The method uses viral transcriptase to prepare the gene and cloning in E. coli plasmids to amplify it. In this manner, full-length, double-stranded globin DNA was synthesized in vitro from rabbit globin mRNA and cloned in E. coli to produce faithful copies of the globin DNA. It should be possible in this way to isolate and amplify any single structural gene starting with a heterogeneous mixture of mRNA's such as those found in differentiating cells (T. Maniatis, et al., 1976).

A correlation between anchorage independence (ability to grow as colonies in vitro) and tumorigenicity in nude mice is shown by many virally transformed cells. Recently, it was demonstrated that cells that lost anchorage dependency and were tumorigenic also showed a high rate of spontaneous aggregation when suspended in an appropriate medium (T. C. Wright, et al., 1977). This technique could become a quick and reliable index of tumorigenicity. It also offers another approach to investigating the relation of cell surfaces to transformation.

Cells of a rat lymphoma induced by Gross virus are rejected by normal, adult rats unless the latter are x-irradiated. However, once having grown in irradiated rats, the cells can grow progressively in normal adult rats and metastasize widely. The alteration in the cells in irradiated rats is ascribed to lost murine leukemia virus surface antigens. These observations are of some theoretical interest, but they also indicate a possible hazard in radiation therapy (H. L. Ioachim, et al., 1977).

Major Areas of Emphasis

In the future the main thrusts of the program will include:

Identification of viral genes responsible for various viral functions, especially transformation.

Biochemical analysis of the interaction of viral genes and cellular genes. Such interaction is a prerequisite for viral transformation. Among the questions asked are: "Does interaction occur at particular sites of the cell's genome?" "Do some sites lend to transformation

but not others?" "What is the nature of the interaction which in some cases leads to transformation but not in others?"

Analysis of the manifold events following integration of tumor virus genes and cellular genes, such as the messages which emanate from this interaction and identification of resulting products, such as proteins, which directly affect cell behavior. Some protein product of the transforming viral gene may be directly responsible for the transforming event. Such proteins are being widely sought. They may be extremely useful in devising procedures to counteract their effects.

Analysis of the structure and function of tumor virus components other than genes. Such studies are important in providing markers for ecological and epidemiological studies of tumor viruses, in determining relationships among them, and in providing potential reagents for diagnosis and active and passive immunization.

Analysis of endogenous viral information. All cells may contain normal cellular constituents related to the transforming genes of tumor viruses. Some types of endogenous "viral information" are inactive in the cells in which they normally exist, but they can readily transform foreign cells. Research is addressing the following questions: "What causes the information to remain suppressed or activated?" "Do chemical carcinogens have a role in this activation?" "Does endogenous viral information have a useful regulatory role in the cell's economy?" "If this endogenous information is truly a normal cell constituent, do tumor viruses function mainly as carriers of this information from one cell to another and from one species to another?"

Analysis of virus-transformed cells. What is the significant alteration of a normal cell which confers malignancy upon it? Many biological, biochemical, and morphological interactions accompany transformation, but which are primary, which secondary, which significant, and which trivial? These questions absorb numerous investigators because the identification of the significant expressions of transformation may suggest fresh approaches to problems in detection, diagnosis, prevention, and treatment of cancer. Investigations to suppress the malignant behavior of virus transformed cells also have a high priority in the Program.

Other Program Activities

The Viral Oncology Program supports workshops and conferences. During fiscal year 1977, those of particular interest were investigator-initiated and included: (1) "Seminars and Workshops in Techniques in Cancer Research" at Aspen, Colorado, covering various aspects of cancer biology, carcinogenesis, viral oncology, and clinical subjects; (2) five workshops at Cold Spring Harbor covering cell culture, animal viruses, molecular biology and genetics of yeast, molecular cytogenetics, and advanced bacterial genetics; (3) a Gordon Research Conference on Cancer at Colby College, and (4) separate

conferences on origins of lymphocyte diversity, parvoviruses, herpesviruses, SV-40 polyoma and adenoviruses, and RNA tumor viruses.

Still other activities included (1) participation in activities of the collaborative Virus Cancer Program such as in-house advisory committees. This interaction is useful in facilitating exchange of information in areas of mutual or overlapping interests and responsibilities; (2) coordination and organization of conferences and workshops on metastasis in connection with the U.S.-Japan Cooperative Cancer Research Program of the NCI; and (3) assistance in evaluating potential program administrators for the National Cancer Institute and other units of the National Institutes of Health as a member of a Civil Service panel.

VIRAL ONCOLOGY PROGRAM

SUMMARY BY SUB-CATEGORY
(Dollars in Thousands)

	NONCOMPETING		COMPETING		TOTAL	
	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT
I. RNA TUMOR VIRUSES						
A. AVIAN	37	$3,513	11	$968	48	$4,481
B. MURINE	29	2,022	10	823	39	2,845
C. BREAST CANCER	4	955	1	153	5	1,108
D. OTHER (HUMAN, CAT, RAT, ETC.)	12	825	1	45	13	870
TOTAL RNA TUMOR VIRUSES	82	7,315	23	1,989	105	9,304
II. DNA TUMOR VIRUSES						
E. POLYOMA	9	777	3	338	12	1,115
F. SV40 AND RELATED VIRUSES	35	2,513	15	1,284	50	3,797
G. HERPES	23	1,633	3	207	26	1,840
H. ADENO	11	749	3	246	14	995
I. OTHER	4	390	0	0	4	390
TOTAL DNA TUMOR VIRUSES	82	6,062	24	2,075	106	8,137
III. RELATED AND SPECIAL PROGRAMS	48	9,860	4	2,291	52	12,151
TOTAL VIRAL ONCOLOGY	212	$23,237	51	$6,355	263	$29,592

REFERENCES

Battula, N. and Temin, H. M., Infectious DNA of Spleen Necrosis Virus Is Integrated at a Single Site in the DNA of Chronically Infected Chicken Fibroblasts. Proc. Natl. Acad. Sci. 74:281-285, 1977. (CA 07175)

Botcham, M., Topp, W., and Sambrook, J., The Arrangement of Simian Virus 40 Sequences in the DNA of Transformed Cells. Cell 9:269-287, 1976. (CA 13106)

Croce, C. M., Assignment of the Integration Site for Simian Virus 40 to Chromosome 17 in GM54VA, A Human Cell Line Transformed by Simian Virus 40. Proc. Natl. Acad. Sci. 74:315-318. (CA 16685)

Friend, C., Sher, W., Holland, J. G., and Sato, T., Hemoglobin Synthesis in Murine Virus Induced Leukemic Cells In Vitro. Stimulation of Erythroid Differentiation by Dimethyl Sulfoxide. Proc. Natl. Acad. Sci. 68:378-382, 1971. (CA 10000)

Gusella, J. F. and Housman, D., Induction of Erythroid Differentiation In Vitro by Purines and Purine Analogues. Cell 8:263-269, 1976. (CA 14051)

Ioachim, H. L., Pearce, A., and Keller, S. E., Antigenic Deletion and Malignant Enhancement Induced in Lymphoma Cells by Passage Through X-Irradiated Hosts. Nature 256:55, 1977. (CA 16997)

Jolicoeur, P. and Baltimore, D., Effect of Fv-1 Gene Product on Proviral DNA Formation and Integration in Cells Infected with Murine Leukemia Viruses. Proc. Natl. Acad. Sci. 73:2236-2240, 1976. (CA 14051)

Kawashima, K., Ikeda, H., Hartley, J. W., Stockert, E., Rowe, W. P., and Old, L. J., Changes in the Expression of Murine Leukemia Virus Antigens and Production of Xenotropic Virus in the Late Preleukemic Period in AKR Mice. Proc. Natl. Acad. Sci. 73:4680-4684, 1976. (CA 08748)

Ketner, G. and Kelly, T. J., Jr., Integrated Simian Virus 40 Sequences in Transformed Cell DNA: Analysis Using Restriction Endonucleases. Proc. Natl. Acad. Sci. 73:1102-1106, 1976. (CA 16519)

Leong, J. C., Kane, J. P., Oleszko, O., and Levy, J. A., Antigen-Specific Nonimmunoglobulin Factor That Neutralizes Xenotropic Virus Is Associated with Mouse Serum Lipoproteins. Proc. Natl. Acad. Sci. 74:276-280, 1977. (CA 13086)

Levy, S. B., Rubenstein, D. B., and Tavassali, M., The Spleen in Friend Leukemia, II. Nonleukemic Nature of Spleen Stroma. J. Natl. Cancer Inst. 56:1189-1195, 1976. (CA 10000)

Maniatis, T., Kee, S. G., Efstratiadis, A., and Kafatis, F. C., Amplification and Characterization of a B-Globin Gene Synthesized In Vitro. Cell 8:163-182, 1976. (CA 13106)

McDonald, H. C. and Ferrer, J. F., Detection, Quantitation, and Characterization of the Major Internal Virion Antigen of the Bovine Leukemia Virus by Radioimmunoassay. J. Natl. Cancer Inst. 57:875-882, 1976. (CA 14193)

Papahadjopoulos, D., Vail, W. J., Jacobson, K., and Poste, G., Cochleate Lipid Cylinders: Formation by Fusion of Unilamellar Lipid Vesicles. Biochem. Biophys. Acta. 394(3):483-491, 1975. (CA 18527)

Poste, G. and Papahadjopoulos, D., Lipid Vesicles as Carriers for Introducing Materials into Cultured Cells: Influence of Lipid Composition and Mechanism(s) of Vesicle Incorporation into Cells. Proc. Natl. Acad. Sci. 73:1603-1607, 1976. (CA 18260)

Prochownik, E. V. and Kirsten, W. H., Nucleic Acid Sequences of Primate Type C Viruses in Normal and Neoplastic Human Tissues. Nature 267: 175-177, 1977. (CA 14898)

Ritze, E., Martin, D. S., Stolfi, R. L., and Spiegelman, S., Plasma Levels of a Viral Protein as a Diagnostic Signal for the Presence of Tumor: The Murine Mammary Tumor Model. Proc. Natl. Acad. Sci. 73:4190-4194, 1976. (CA 02332)

Rothenberg, E., Smotkin. D., Baltimore, D., and Weinberg, R. A., In Vitro Synthesis of Infectious DNA of Murine Leukemia Virus. In press. (CA 14051)

Stacy, D. W., Allfrey, V. G., and Hanafusa, H., Microinjection Analysis of Envelope Messenger Activities of Avian Leukosis Viral RNA's (Microinjection Oncornavirus/Messenger RNA). In press. (CA 14935)

Stehlin, D., Varmus, H. E., Bishop, J. M., and Vogt, P. K., DNA Related to the Transforming Gene(s) of Avian Sarcoma Viruses Is Present in Normal Avian DNA. Nature 260:170-173, 1976. (CA 12705)

Sveda, M. M. and Soeiro, R., Host Restriction of Friend Leukemia Virus: Synthesis and Integration of the Provirus. Proc. Natl. Acad. Sci. 73:2356-2380, 1976. (CA 70580)

Wang, L. H., Duesberg, P. Mellon, P., and Vogt, P., Distribution of Envelope Specific and Sarcoma-Specific Nucleotide Sequences from Different Parents in the RNA's of Avian Tumor Virus Recombinants. Proc. Natl. Acad. Sci. 73:1073-1077, 1976. (CA 11426)

Welsh, R. M., Jr., Jensen, F. C., Cooper, N. R., and Oldstone, M. B. A., Inactivation and Lysis of Oncornaviruses by Human Serum. Virology 74:432-440, 1976. (CA 14692)

Wright, T. C., Ukena, T. E., Campbell, R., and Karnovsky, M. J., Rates of Aggregation, Loss of Anchorage Dependence and Tumorigenicity of Cultured Cells. Proc. Natl. Acad. Sci. 74:258-262, 1977. (CA 14723)

NATIONAL ORGAN SITE PROGRAMS BRANCH

The National Organ Site Programs consist of grant-supported National Projects of targeted cancer research. Each Project is a planned research effort oriented toward cancer at a specific organ site. Currently there are National Organ Site Projects concerned with cancers of the urinary bladder, large bowel, pancreas, and prostate. The planning, direction, and coordination of each Project are provided at a Headquarters institution other than the NCI. A National Project Director, who is not an employee of the NCI, is assisted in planning and administration by a Headquarters staff and by a Working Cadre of active research scientists recruited from institutions throughout the Nation. Grant applications are received by the Headquarters and are reviewed by the Working Cadre and by the National Cancer Advisory Board. Applications which are judged scientifically meritorious and relevant to the aims of the Project are recommended to the NCI for funding.

Each National Project Director is a recognized clinical or laboratory scientist with a strong interest in, and professional identification with, a specific organ site cancer. The National Bladder Cancer Project is under the direction of Dr. Gilbert H. Friedell, St. Vincent Hospital, Worcester, Massachusetts; the National Large Bowel Cancer Project, Dr. Murray M. Copeland, M. D. Anderson Hospital and Tumor Institute, Houston, Texas; the National Pancreatic Cancer Project, Dr. Isidore Cohn, Jr., Louisiana State University School of Medicine, New Orleans, Louisiana; and the National Prostatic Cancer Project, Dr. Gerald P. Murphy, Roswell Park Memorial Institute, Buffalo, New York.

In March 1974, the National Cancer Advisory Board approved support for developing information on current research and research opportunities in head and neck cancer. In addition to planning meetings, a group of experts held a Workshop on Head and Neck Cancer February 16 to 18, 1976, in New Orleans, Louisiana.

NATIONAL BLADDER CANCER PROJECT

Description

The National Bladder Cancer Project is a coordinated research effort which sponsors and encourages basic and clinical research directed toward improving techniques for detecting bladder cancer, finding better methods of treating the disease, preventing exposure to carcinogenic agents, and developing means of protecting high-risk populations.

With the control of bladder cancer as the ultimate goal, a multidisciplinary program plan has been developed to stimulate research in new fields important for bladder cancer. Etiological information is being obtained through epidemiological research, encompassing descriptive, analytical, and experimental approaches. Knowledge of the demographic, biological, and environmental factors associated with the disease permits identification of groups at high risk. Investigating the natural history of the disease includes work on carcinogenic mechanisms, host response and resistance, and premalignant markers. Developing methods to detect and diagnose the disease at an early stage is an important aspect of the research effort. This emphasis assumes that the earlier the diagnosis, the more favorable the prognosis and the more effective the treatment. Bladder carcinoma includes a heterogeneous group of tumors, with varying degrees of malignancy. Various methods of classifying and staging the disease are being evaluated to enable the most appropriate therapy. To improve results of treatment, several avenues of clinical research are being pursued, including adjuvant therapy, radiation, chemotherapy, and immunotherapy.

Accomplishments

The development of an *in vitro* model system for investigating carcinogenic factors in bladder cancer is progressing. The first successful long-term growth and passage of normal rat urothelial cells in culture has been made possible by the following modifications in technique (Roszell, Douglas, and Irving, 1977). One modification allows a collagenase-trypsin mixture to penetrate the epithelial layer; this disaggregates the cells with minimal damage and maximal viability. A second modification involves an addition to the culture medium of the polyamines putrescine, spermine, and spermidine at initiation or transfer of the culture. These new culture methods result in a supply of normal rat urothelial cells which can be used for studies of carcinogenesis, and for controls in many types of experiments on tumor cells.

Work is continuing on techniques to identify early bladder cancer lesions through cytologic screening. In a study of over 25,000 urologic outpatients, 2.2 cases per 1,000 without previous bladder neoplasm had urine cytology positive for urothelial cancer, which was confirmed by subsequent biopsies although no lesion could be seen at cystoscopy (Farrow, Utz, and Rife, 1976). In 21 of these cases treated by total cystectomy, *in situ* transitional

cell carcinoma of Grade 3-4 involved over one-third of the mucosa in 70 percent with distal ureteral involvement in 57 percent, urethral invasion in 81 percent, and extension into the prostatic ducts in 37 percent.

Urinary cytology is being automated, utilizing acridine orange stain and flow cytofluorometry (Melamed, Traganos, Sharpless, and Darzynkiewicz, 1976). Double-stranded DNA yields a green fluorescence from intercalated dye, while single-stranded RNA or melted DNA causes a red fluorescence to be produced by bound stacked dye. Epithelial cells are readily distinguished from squamous cells and leukocytes. Although benign and malignant cells exhibit differences in DNA/RNA ratios, much overlap between the two populations is found on multiparameter display. A promising alternative being studied is to remove the RNA with RNAse and examine for differences in DNA melting that indicate structural differences in nuclear chromatin. In this way, clear-cut separation between populations of cultured normal and tumor cells is found when they are heated at 90° for five minutes prior to staining (Melamed, Darzynkiewicz, Traganos, and Sharpless, 1977).

Emphases and Projections

In human urinary-tract infections, dimethylnitrosamine can be produced in appreciable quantities by reaction of nitrite reduced from nitrate by pathogenic organisms together with dimethylamine normally present in urine. Antibiotic therapy is found to suppress promptly both the infection and the nitrosamine formation. Since dimethylnitrosamine requires tissue activation, a double-headed search is under way for the presence of such activating enzymes in bladder mucosa and for the presence of naturally occurring precursors of nitroso compounds which do not require activation.

The prognostic usefulness of karyotypic analysis in bladder cancer has been demonstrated in a study of 62 papillary tumors. Twelve benign tumors were totally diploid. Of the remaining 50 noninvasive papillary cancers, 32 with marker chromosomes gave 11 recurrences within a year, whereas 18 without marker chromosomes had only one recurrence. Therefore, the presence of a marker chromosome in a papillary tumor carries a pessimistic prognosis. In advanced invasive cancer, the karyotypic picture is near-triploid with four or more marker chromosomes in evidence.

Improved prognostic capability through immunology may be provided by the delayed hypersensitivity reaction to dinitrochlorobenzene (DNCB) using skin tests. The percentage of negative tests rose with advancing disease, while removal of tumor increased positive reactions from 20 percent to 40 percent. It appears that radiation therapy can cripple the immune defenses at the same time it is attacking tumor cells, since negative DNCB skin tests rose from a pretreatment level of 39 percent to a postradiation level of 96 percent.

In studies of the course of bladder cancer under accepted methods of treatment in nine hospitals, it has been found that 30 percent of patients with initial lesions will have recurrence within a year, while those who had a previous lesion have a recurrence rate of 70 percent within a year.

In identifying in situ lesions, a study of cells in bladder washings at the time of bladder removal is more effective than a study of cells in urine. It has further been found that 30 percent of patients with bladder cancer will show atypical cells in selected superficial biopsies of uninvolved bladder epithelium near in situ lesions. Studies are now being conducted to determine the relative prognosis of patients with in situ carcinoma or atypia at sites in addition to an identified cancer lesion as compared to those patients without such additional changes. The clinical trial to assess the curative and prophylactic effects of thio-TEPA introduction into the bladder will be continued. No new patients will be entered into the program comparing the effectiveness of definitive radiotherapy with that of radiotherapy followed by appropriate surgery, because unpublished data from England have indicated the general superiority of the latter method.

Investigators at the National Institutes of Health have shown that 13-cis-retinoic acid, an analog of vitamin A, inhibits the formation of bladder lesions in animals fed known carcinogens. This compound is relatively low in toxicity in humans, and will be employed in clinical trials being developed for patients who have had single, multifocal, or recurrent bladder tumors removed by surgery. It will be seen if retinoid administrations will significantly reduce future recurrence of cancer in these three groups of patients.

Studies are being developed to conduct adjuvant trials using cis-diammine-dichloroplatinum (II) in those patients who have had radiation and surgical removal of the bladder. Chemicals which have been shown to be effective in treating tumors in other organs of the body will be tested in patients with advanced cancer of the bladder.

A computer model of the bladder cancer disease process is being developed. The model will represent the natural history of the disease and the potential influence of various treatments. It will provide a means for the design and comparison of treatment strategies and will be used to help identify research areas where critically needed data are lacking.

Other Program Activities

A National Bladder Cancer Conference was held in Miami from November 28 to December 1, 1976. The total attendance was 300. Five sessions were held: Assessment and Management of Deeply Invasive and Metastatic Lesions, Recognition of Early Bladder Cancer and Premalignant Epithelial Changes, Classification of Tumor and Host, Management of Early Bladder Lesions, and Cause and Prevention of Bladder Cancer. The proceedings will be published as a supplement to the August issue of Cancer Research.

A mini-workshop was held the evening of May 18, 1977, at the Denver meeting of the American Association for Cancer Research on the topic of carcinogenesis in bladder cancer.

NATIONAL BLADDER CANCER PROJECT

(Dollars in Thousands)

NONCOMPETING		COMPETING		TOTAL	
NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT
27	$2,468	15	$1,460	42	$3,928

REFERENCES

Cohen, S. M., Jacobs, J. B., Arai, M., Johansson, S., and Friedell, G. H., Early Lesions in Experimental Bladder Cancer. 1. Experimental Design and Light Microscopic Findings. Cancer Res. 36:2508-2511, 1976. (CA 15945)

Cooper, T. P., Wheelis, R. F., Correa, R. J., Gibbons, R. P., Mason, J. T., and Cummings, K. B., Random Mucosal Biopsies in the Evaluation of Patients With Carcinoma of the Bladder. J. of Urol. 117:46-48, 1977. (CA 17466)

Farrow, G. M., Utz, D. C., and Rife, C. C., Morphological and Clinical Observations of Patients with Early Bladder Cancer Treated with Total Cystectomy. Cancer Res. 36:2495-2501, 1976. (CA 16725)

Friedell, G. H., Carcinoma, Carcinoma In Situ, and "Early Lesions" of the Uterine Cervix and Urinary Bladder: Introduction and Definitions. Cancer Res. 36:2482-2484, 1976. (CA 15490)

Hakala, T. R., Lange, P. H., Elliott, A. Y., and Fraley, E. E., Changes in Cell-Mediated Cytotoxicity During the Clinical Course of Patients with Bladder Carcinoma. J. of Urol. 115:268-273, 1976. (CA 15551)

Heney, N. M., Szyfelbein, W. M., Daly, J. J., Prout, G. R., and Bredin, N. C., Positive Urinary Cytology in Patients without Evident Tumor. J. of Urol. 117:223-224, 1977. (CA 15944)

Herr, H. W., Bean, M. A., and Whitmore, W. F., Jr., Decreased Ability of Blood Leukocytes from Patients with Tumors of the Urinary Bladder to Act as Stimulator Cells in Mixed Leukocyte Culture. Cancer Res. 36:2754-2760, 1976. (CA 19165)

Hopp, M. L., Matsumoto, M., Lee, C., and Oyasu, R., Absorption and Protein Binding of N-2-Fluorenylacetamide and Its Metabolites in the Bladder of the Rabbit. J. Nat. Cancer Inst. 58:281-285, 1977. (CA 14649)

Jacobs, J. B., Arai, M., Cohen, S. M., and Friedell, G. H., Early Lesions in Experimental Bladder Cancer, 2. Scanning Electron Microscopy of Cell Surface Markers. Cancer Res. 36:2512-2517, 1976. (CA 15945)

Jacobs, J. B., Arai, M., Cohen, S. M., and Friedell, G. H., Light and Scanning Electron Microscopy of Exfoliated Bladder Epithelial Cells in Rats Fed N-[4-(5-Nitro-2-Furyl)-2Thiazolyl] Formamide, J. Nat. Cancer Inst. 57:63-66, 1976. (CA 15945)

Jacobs, J. B., Cohen, S. M., Arai, M., Friedell, G. H., Bulay, O., and Urman, H. K., Chemically Induced Smooth Muscle Tumors of the Mouse Urinary Bladder. Cancer Res. 36:2396-2398, 1976. (CA 15945)

King, C. M., Traub, N. R., Cardona, R. A., and Howard, R. B., Comparative Adduct Formation of Arylamine Derivatives with Macromolecules of Isolated Liver Parenchymal Cells, Cancer Res. 36:2374-2381, 1976. (CA 15640)

Koss, L. G., Nakanishi, I., and Freed, S. Z., Nonpapillary Carcinoma In Situ and Atypical Hyperplasia in Cancerous Bladders: Further Studies of Surgically Removed Bladders by Mapping. Urol. 9:442-455, 1977. (CA 15803)

Melamed, M. R., Darzynkiewicz, Z., Traganos, F., and Sharpless, T. K., Nucleic Acid Content and Nuclear Chromatin Structure of Human Bladder Cell Culture Lines as Studied by Flow Cytofluorometry. Cancer Res. 37:1227-1231, 1977. (CA 14134)

Melamed, M. R., Traganos, F., Sharpless, T., and Darzynkiewicz, Z., Urinary Cytology Automation--Preliminary Studies with Acridine Orange Stain and Flow-Through Cytofluorometry. Invest. Urol. 13:331-338, 1976. (CA 14134)

Oyasu, R., Manning, D. J., Matsumoto, M., and Hopp, M. L., Heterotopic Urinary Bladder with a Communicating Reservoir. Cancer Res. 36: 2261-2267, 1976. (CA 14649)

Roszell, J. A., Douglas, C. J., and Irving, C. C., Polyamine-stimulated Growth of Cultured Rat Urinary Bladder Epithelial Cells. Cancer Res. 37:239-243, 1977. (CA 16765)

Wang, C. Y., Benson, R. C. Jr., and Brian, G. T., Mutagenicity for Salmonella typhimurium of Urine Obtained from Humans Receiving Nitro-Furantoin. J. Nat. Cancer Inst. 58:817-819, 1977. (CA 17449)

NATIONAL LARGE BOWEL CANCER PROJECT

Description

The National Large Bowel Cancer Project sponsors and encourages interdisciplinary basic and clinical research directed toward the prevention, control, and cure of large bowel cancer. In the pursuit of these objectives a broad spectrum of programs has been developed to: identify causes and inhibitors of large bowel cancer; identify and clarify biochemical and molecular controls of such cancers; identify individuals at high risk; develop methods of early diagnosis; develop a means for colon cancer prevention; apply modern pharmacologic methods to develop new chemotherapeutic treatments; clarify the immunobiology of colorectal cancers; and apply immunobiological methods to prevent and treat colon cancer.

Accomplishments

Work is progressing on the identification of causes and inhibitors of large bowel cancer. Metabolic and epidemiologic studies have implicated dietary fat and beef in the etiology of large bowel cancer (Wynder, Reddy et al., 1976). Several colon carcinogens have been identified: 1,2-dimethylhydrazine (DMH), azoxymethane and methylazoxymethanol (MAM), N-methyl-N-nitro-nitrosoguanidine and methylnitrosourea (Bralow and Weisburger, 1976). Isotopically labeled DMH is metabolized to azomethane, which is oxidized to azoxymethane and then hydroxylated to methylazoxy-methanol, an ultimate colon carcinogen (Fiala, 1977). A number of complex interrelationships have been demonstrated between N-methyl-N-nitrosoguanidine, a potent ultimate colon carcinogen, and guanylate cyclase in colonic epithelial cells (DeRubertis and Craven, 1977). The colon carcinogenic effects of DMH are enhanced by polyunsaturated fats. Thymocytes of rats fed polyunsaturated fats respond poorly to mitogens (Broitman, 1977). Lithocholic acid and deoxycholic acid which are increased in the feces of humans on high fat-beef diets augment the colon carcinogenic effects of MNNG. Several antioxidants (disulfiram, sodium diethylthiocarbamate, bis (ethylxanthogen), N-ethylmaleimide, and selenium) have been demonstrated to inhibit the carcinogenic effects of certain colon carcinogens (Wattenberg, Loub, Lam, and Speier, 1976; DeRubertis, 1977; Jacobs, Jansson, and Griffin, 1976).

Several studies are involved in the identification of biochemical and molecular controls. Cell membrane variants have been identified in colon cancer cells, e.g., blood group A is deleted. Changes in glycopeptides and glycolipids, decrease in glycosyl transferase, and a unique galacto protein with a significant decrease in fucose and a molecular weight of 200,000 have been observed (Kim, Tsao, and Whitehead, 1977). Two unique DNA-associated non-histone proteins have been identified in colon cancers in humans and in DMH-treated rats. These non-histone proteins, which are unique to colon cancer, can also be demonstrated in normal-appearing colonic mucosa of rats within 4 weeks of beginning treatment with DMH (Boffa, Vidali, and Allfrey, 1976; Boffa and Allfrey, 1977). Enzyme variants have been identified in cancers of the large bowel. Importantly, a variant of adenosine deaminase

has been identified in adenocarcinomas of the colon but not in an epidermoid cancer of the anus (Balis, 1976; Trotta and Balis, 1977).

Segments of the population at high risk for large bowel cancer are under study. Markers have been demonstrated in members of families with precancerous heritable polyposis of the colon. Tetraploidy has been observed in cultured epithelial cells (skin and colon) of patients with Gardner's syndrome (Danes, 1976) and surface labeling with tritiated thymidine has been observed in polyps and normal appearing flat mucosal cells (Deschner, Long, and Winawer, 1976). Undegraded cholesterol has been demonstrated in feces of patients with heritable polyposis (Reddy, Mastromarino, et al., 1976).

Early diagnosis is an important factor in the control and cure of large bowel cancer. Screening asymptomatic individuals with the hemoccult slide technique resulted in positive tests for fecal blood in 1 percent of the test subjects. Workup of 82 individuals with positive tests for fecal blood resulted in the detection of 11 colon cancers, 10 of which were localized; and one or more colon polyps were detected in 32 of these patients. CEA activity in colonic washings is elevated in most patients with large bowel polyps or with colon cancer. Normal patients and patients with small polyps had low CEA levels in the colonic washings (Winawer, Schottenfeld, Miller et al., 1976; Winawer, Fleisher, Green et al., 1976).

Modern pharmacologic and immunobiologic methods are being applied in developing new chemotherapeutic treatments for large bowel cancer. Four murine models of transplantable colon cancer have been developed for testing the anticolon tumor effects of established and new chemotherapeutic agents. Anguidine is an effective antitumor agent against one of the colon cancer lines. A combination of anguidine and 5 FU can cure animals bearing one of the colon cancers. Other effective anticolon tumor agents include MeCCNU, 5FUdR, and CIS-platinum II. Active combinations include MeCCNU and 5 FU, adriamycin and 5 FU, and cyclophosphamide and procarbazine (Corbett and Griswold, 1976; Corbett, Griswold et al., 1977). Rats immunized with colon tumor extracts reject colon tumor transplants. The administration of DMH to rats previously immunized with colon tumor extracts does not result in colon carcinogenesis (Sjogren, 1977). One study indicates that adjuvant immunotherapy with BCG decreases the rate of recurrence in patients with Dukes' C cancers of the large bowel.

Emphasis and Projections

The National Large Bowel Cancer Project encourages research in carcinogenesis, epidemiology, cell kinetics, molecular controls of cellular proliferation and maturation, early diagnosis, human genetics, and immunobiology of colorectal cancers. Recently, animal models which might be used in developing new chemotherapeutic agents have become available. Animal models of transplantable large bowel cancer, xenografts, and *in vitro* cell lines of human colon cancer are also available for new experimental studies of pharmacology and experimental chemotherapy of large bowel cancer.

Epidemiologic studies have implicated dietary fat and beef in the etiology of large bowel cancer. Antioxidants such as disulfiram, sodium diethylthiocarbamate, bis (ethylxanthogen), and N-ethylmaleimide have been demonstrated capable of inhibiting the action of certain colon carcinogens. In addition, the administration of 13 CIS retinoic acid, an analogue of vitamin A, or the administration of Lactobacillus acidophilus inhibits the colon carcinogenic effects of DMH. Selenium administered in the drinking water inhibits the colon carcinogenic effects of DMH and of methylazoxymethanol. The NLBCP is promoting research in colon cancer prevention, based on these observations and conducted on individuals at high risk for large bowel cancer.

The availability of xenografts of human colon cancer in immune-deficient mice makes it possible to promote pharmacological studies on the chemotherapy of human colon cancers. A grantee has demonstrated that sodium cyanate inhibits protein synthesis by DMH-induced colon cancers in rats but not of normal colon mucosa. Furthermore, this agent does not interfere with protein synthesis by in vitro cultured cell lines of colon cancer. These studies will be expanded.

The National Project proposes to collaborate with the Gastrointestinal Tumor Study Group of the Division of Cancer Treatment in promoting clinical trials and programs of adjuvant therapy with anguidine alone or in combination with other antitumor agents in the management of patients with large bowel cancer.

To identify colon cancer causative agents, research is being encouraged to isolate and identify mutagens in the urine and feces of animals under treatment with colon carcinogens, in patients at risk for colon cancer, and in human subjects on diets thought to increase the risk for such cancers.

Other Program Activities

In January 1977, the National Large Bowel Cancer Project sponsored its third major Workshop on Large Bowel Cancer. The theme was Modern Methods of Biomedical and Clinical Research Toward Control and Cure of Large Bowel Cancer. The Workshop was attended by 193 biomedical and clinical investigators committed to the objectives of the National Large Bowel Cancer Project. An important feature of the Workshop was promotion of interdisciplinary communication and cooperation of biochemists, cell biologists, cancer biologists, immunologists, pharmacologists, and clinical investigators in research on the biology, control, and prevention of large bowel cancer.

NATIONAL LARGE BOWEL CANCER PROJECT

(Dollars in Thousands)

NONCOMPETING		COMPETING		TOTAL	
NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT
45	$4,227	11	$1,046	56	$5,273

REFERENCES

Balis, M.D., Enzymatic Aspects of Intestinal Epithelium. Sem. Oncol. 3:337, 1976. (CA 14906)

Boffa, L. D. and Allfrey, V. G., Changes in Chromosomal Proteins in Colon Cancer: The Complexity and DNA Binding Properties of Tumor Associated Proteins and Evidence for Their Association with the Malignant State in Human Colonic Epithelium. Cancer. In press. (CA 22419)

Bralow, S. P. and Weisburger, J. H., Experimental Carcinogenesis. Clinics in Gastroenterology 5:527, 1976. (CA 15400)

Corbett, T. H., Griswold, D. P. Jr., Roberts, B. J., Peckham, J. C., and Schabel, F. M., Jr., Evaluation of Single Agents and Combinations of Chemotherapeutic Agents in Mouse Colon Carcinomas. Cancer. In press. (CA 17303)

Danes, B. S., Increased Tetraploidy: Cell Specific for the Gardner Gene in the Cultured Cell. Cancer 38:1983, 1976. (CA 15973)

Deschner, E., Long, F., and Winawer, S. J., Isotopically Labeled Colonic Cytology in Mice and Men. Gastroenterology 70:877, 1976. (CA 15429)

DeRubertis, F. R. and Craven, P. A., Calcium-Independent Modulation of Cyclic GMP and Activation of Guanylate Cyclase by Nitrosamines. Science 193:897, 1976. (CA 16908)

DeRubertis, F. R., Nitrosamines are Agonists of Guanylate Cyclase in Colonic Mucosa and Other Tissues. Cancer. In press. (CA 16908)

Fiala, E. S. and Weisburger, J. H., Investigations into the Metabolism and Mode of Action of the Colon Carcinogens 1,2-dimethylhydrazine and Azoxymethane. Cancer. In press. (CA 15400)

Jacobs, M. M., Jansson, B., and Griffin, A. C., Inhibitory Effects of Selenium on 1,2-dimethylhydrazine and Methylazoxymethanol Acetate Induction of Colon Tumors. Cancer Letters 2:133, 1976. (CA 11430)

Kim, Y. S., Tsao, D., and Whitehead, J., Biology of Colon Cancer: An Overview. Cancer. In press. (CA 14905)

Reddy, B. S., Mastromarino, A., Gustafson, C., Lipkin, M., and Wynder, E. L., Fecal Bile Acids and Neutral Sterols in Patients with Familial Polyposis. Cancer 38:1694, 1976. (CA 18034)

Sjogren, H. O., The Application of Immunobiology to the Development of Immunotherapeutic Programs for Patients with Large Bowel Cancer. Cancer. In press. (CA 14924)

Trotta, P. P. and Balis, M. E., Enzyme Variants in Large Bowel Cancer. Cancer. In press. (CA 14906)

Wattenberg, L. W., Loub, W. D., Lam, L, K., and Speier, J. L., Dietary Constituents Altering the Responses to Chemical Carcinogens. Fed. Proc. 35:1327, 1976. (CA 15638)

Winawer, S. J., Schottenfeld, D., Miller, D., Sherlock, P., Deschner, E., Stearsn, M., Watson, R., Edelman, M., Fleisher, M., Schwartz, M., Hajdo, S., and Melamed, M. In Proc. Third Internatl. Symp., Detection and Prevention of Cancer, H. Nieburgs (ed.)., Marcell Dekker, Inc., New York, N. Y., 1976. (CA 15429)

Winawer, S. J., Fleisher, M., Green, S., Bhargava, D., Boyle, C., Sherlock, P., and Schwartz, M. K., Carcinoembryonic Antigen in Colonic Lavage. Gastroenterology. In press. (CA 15429)

Wynder, E. L., Reddy, B. S., McCoy, G. D., Weisburger, J. H., and Williams, G. M., Diet and Cancer of the Gastrointestinal Tract. Adv. Inter. Med. 22:397, 1976. (CA 15400)

NATIONAL PANCREATIC CANCER PROJECT

Description

The National Pancreatic Cancer Project, now in its third year, is well established and increasingly active. To date, 110 grant applications have been received in the areas of diagnosis, epidemiology, experimental biology, immunology, pathology, and treatment of pancreatic cancer. Research projects underway involve tissue culture of bovine pancreas for use in carcinogenesis work; pancreatic metabolism of selected carcinogens; improvements in techniques of radiological diagnosis, scanning, and ultrasonography; immunobiology and immunodiagnosis; etiology; biochemistry of pancreas cell differentiation; endocrinology; and pathology. The National Project continues to solicit applications for research in biology, etiology, epidemiology, diagnosis, surgery, radiotherapy, pathology, immunobiology, immunoprophylaxis, immunotherapy, and multimodality treatment.

Accomplishments

Four projects involve studies of immunologic mechanisms in relation to pancreatic cancer. Chu et al (CA 18410) are studying oncofetal antigens in primary tumor, metastases, and various body fluids. Three such antigens have been identified, and development of antisera is partially completed. Two other studies are attempting to identify antigens by a number of new techniques utilizing both human pancreatic cancer and human fetal pancreas (Turner, CA 19193; Mihas, CA 19196). In the fourth study, an investigator is attempting to delineate which human tissues or tumors cross-react with antiserum to human pancreatic cancer (Klavins, CA 21028). All of these studies are directed at developing a highly specific immunodiagnostic test for pancreatic cancer.

Two major studies are under way in epidemiology of pancreatic cancer. One is an attempt to identify groups of individuals at high risk by a case-control study of pancreatic cancer patients and neighborhood controls with detailed questionnaires (Mack, CA 19171). The other is a study aimed at delineating geographic areas--by county, state, etc.--which have high or low rates of pancreatic cancer. There will be an attempt to relate geographic differences with specific risk factors (Sauer, CA 19212).

A study by Githens and his co-workers is attempting to isolate pancreatic duct cells so that the effects of various carcinogenic agents on this epithelium can be evaluated effectively (CA 19177). In another study, cultures of bovine pancreatic ductal explants are subjected to various carcinogens to determine the effects of these agents (Jones, CA 19197). Hyperplastic nodules have been observed to appear prior to the development of pancreatic cancer in rats and this has been demonstrated in a study by Shinozuka et al. (CA 20116).

Attempts are being made to define the importance of these nodules, as well as the sequence of events leading to their development. In another study,

pancreatic juice and bile are collected to determine excretion rates of carcinogens suspected to be in these fluids (Epstein, CA 20914). Varricchio and co-workers (CA 19584) are investigating the specific sites of F1 histone phosphorylation during the early stages of pancreas regeneration and carcinogenesis. This work is looking at the role of histone phosphorylation in carcinogenesis and development.

A new radiologic technique for early diagnosis of pancreatic lesions is being evaluated in animals and man. The technique involves injection of dye and radiopaque medium into the pancreatic venous system. Its feasibility has been demonstrated, although safety and other factors are still being explored (Rosch, CA 19221). Another study utilizes a battery of laboratory, radiologic, endoscopic, and immunologic tests on suspected cases of pancreatic cancer, with later comparisons of relative accuracy (McDermott, CA 19232). Goodale and associates (CA 20115) are investigating a technique for obtaining cells and pancreatic secretions from pancreatic ducts for histologic study. Pancreatic secretions are also obtained for immunologic and other studies. Still another study (Harbert, CA 19397) compares the results of ACTA-scans, performed on patients with known or suspected pancreatic carcinoma, with other noninvasive diagnostic techniques.

Emphases and Projections

The National Pancreatic Cancer Working Cadre is reevaluating the Project's goals in diagnosis, treatment, epidemiology, and pathology. Small working groups aided by consultants, are defining research needs and funding projections. The Working Cadre is developing plans for multi-institutional clinical studies of the diagnosis and treatment of pancreatic cancer. The Working Cadre believes that cooperative studies are essential in these two areas.

Other Program Activities

The National Project sponsored a meeting with the American Pancreatic Study Group in Chicago, November 4 to 5, 1976. The meeting included presentations by eleven grantees of the Project, and invited talks summarized current research in several areas of pancreatic cancer. Another meeting with the American Pancreatic Study Group is planned for November 3 to 4, 1977.

The National Project has initiated a newsletter which keeps grantees and workers in the field informed of progress being made by the Project, and provides information on currently funded research. A bibliography of publications relating to pancreatic cancer has been initiated and will appear, updated, in each issue of the newsletter.

NATIONAL PANCREATIC CANCER PROJECT

(Dollars in Thousands)

NONCOMPETING		COMPETING		TOTAL	
NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT
24	$1,350	8	$569	32	$1,919

REFERENCES

Chu, T. M., Holyoke, E. D., and Douglass, H. O., Isolation of a Glycoprotein from Ascites Fluid of Pancreatic Carcinoma. Can. Res. In press, 1977. (CA 18410)

Cohn, I. Jr., The National Pancreatic Cancer Project: An Update, J. Surg. Oncol. 9:49-51, 1977. (CA 17500)

Harvey, S. R., Van Dusen, L. R., Howell, J., Goldrosen, M., and Chu, T. M., Immunochemical Studies on the Onco-Fetal Properties of Carcinoembryonic Antigen. Onco-Developmental Gene Expression, Academic Press, p. 587, 1976. (CA 18410)

Mack, T. M. and Henderson, B. E., Cancer Registries for General and Selected Uses. US-USSR Joint Monograph on Cancer Epidemiology. In press, 1977. (CA 19171)

Mack, T. M., Pike, M. C., and Casagrande, J. T., Epidemiologic Methods for Human Risk Assessment. In Origins of Human Cancer, Cold Spring Harbor Symposium. In press, 1977. (CA 19171)

Shinozuka, H., Topp, J. A., and Kanishi, Y., Ultrastructure of Atypical Acinar Cell Nodules in Rat Pancreas Induced by 4-Hydroxyaminoquinaline-1-oxide. Lab Invest. 34:501-509, 1976. (CA 20116)

Varricchio, F., Postnatal Increase In Histone H1a in the Rat Pancreas. Arch. Biochem. Biophys. In press, 1977. (CA 19584)

Varricchio, F. and Jamieson, G., Reactivity of Heterogeneous F1 Histones with Glutaraldehyde and Formaldehyde. Exp. Cell Res. In press, 1977. (CA 19584)

Wiggans, G., Schein, P. S., MacDonald, J. S., Schelinger, D., and Harbert, J. C., Computerized Axial Tomography for Diagnosis of Pancreatic Cancer. Lancet 2:233-235, 1976. (CA 19397)

NATIONAL PROSTATIC CANCER PROJECT

Description

The overall strategy of the National Prostatic Cancer Project is the development and implementation of a multidisciplinary investigator-initiated research program, both experimental and clinical. Research is aimed at the prevention of prostatic cancer and at decreased morbidity and mortality from this disease. A national plan of research has been developed within which priorities have been identified in etiology/prevention, detection/diagnosis, and treatment.

Active projects that are currently supported in the etiology/prevention area include epidemiologic studies probing socioeconomic, sexual, clinical, and other differences between high- and low-risk groups for prostatic cancer. Etiological roles of dietary, socioeconomic, occupational, sexual, and medical factors are being studied. Other studies demonstrate the presence of viral particles and antigens, and this leads to the investigation of the possible relation of viruses to prostatic cancer. The relation of prostatic carcinoma antigens to cell-mediated immunity is also under study.

Considerable importance is attached to the development of models both *in vitro* and *in vivo*. Projects are currently funded to develop techniques for the separation, isolation, and primary culture of prostatic epithelial cells, both normal and malignant. The development of animal models is actively under way. In addition to their significance for etiologic studies, these models can be used to screen new chemotherapeutic agents.

Major efforts in detection and diagnosis are directed toward the immunologic and biochemical identification of prostatic cancer at its onset. The development of a specific and sensitive radioimmunoassay for prostatic acid phosphatase is under way. Antigenic specificity of normal prostatic tissue has been demonstrated in a variety of species including man, and the National Project is supporting studies on antigenic components of prostatic carcinoma. Studies are also in progress aimed at establishing morphological criteria for discriminating borderline cases, and to establish grading criteria for diagnosis and prognosis.

A major thrust of the National Prostatic Cancer Project has been in the treatment area. Clinical cooperative trials were initiated in 1973. This represented the first nationally randomized study of single chemotherapeutic agents on Stage D patients who fail to respond or no longer respond to conventional treatment. These are patients with histologically confirmed disease and distant metastases. The National Project also supports radiotherapy studies comparing extended field treatment--that is, irradiation--to areas of known involvement plus contiguous lymph nodes, with local field treatment involving irradiation limited to known areas of tumor. Control trials directed at determining the specific efficacy of radiotherapy are also under way.

Accomplishments

An epidemiologic study comparing the high-risk black population in the United States with the low-risk black population in Nigeria is in progress, and there have been a number of significant observations (Jackson, M. A., et al., 1977). In comparing American and Nigerian black patients in the two age groups with most cases (age groups 55-64 and 65-74), there was a marked predominance of grade I carcinoma of the prostate over grades II and III in American blacks. However, this was not noted for Nigerian males. A comparison of the mean ages of black patients with grades I-III carcinoma revealed that the mean ages of patients for all grades were higher in American black males than in Nigerian males. The difference in age distribution of black males with carcinoma of the prostate from Ibadan, Nigeria, and Washington, D.C., may be related to the different age structure of the populations. The preponderance of cases with lower grades in the American patients may be related to earlier diagnosis. Mean blood testosterone and estradiol levels were significantly lower in patients with carcinoma of the prostate from Ibadan, Nigeria, as compared to cancer patients in Washington, D.C. Among Nigerians, the testosterone blood level was significantly higher in controls than in cancer patients. However, no significant differences were found in this parameter between controls and patients in Washington, D.C. There was a significant difference in carcinoma rate between the United States (18.14/100) and Nigeria (6.92/100). No evidence of a significant difference in the distribution of type of carcinoma by age in the United States and Nigeria could be detected.

A significant number of prostatic cancer patients are first seen at an advanced stage of the disease when little can be done for the patient. Studies to detect this disease at an earlier stage are in progress. Serum acid phosphatase measurement by conventional methods of analysis has been the standard probe for detecting the disease and for monitoring patient progress during treatment. These conventional methods have proven inaccurate, insensitive, and nonspecific for acid phosphatase of prostate origin. Two immunological methods for measuring prostatic acid phosphatase have been developed which in preliminary studies have proven accurate, sensitive, and specific (Choe, et al., 1977; Chu, T. M., et al., 1977a; and Chu, T. M., et al., 1977b). A screening program has been initiated in 25-30 institutions throughout the country to further determine the efficacy of immunological assays in the early detection of prostatic cancer and in the monitoring of patient progress.

Over 600 patients have been randomized to eight chemotherapy protocol studies in seven participating insitutions throughout the United States. The different protocols are designed to evaluate single and combined chemotherapeutic agents in patients with histologically proven advanced (Stage D) prostatic cancer. These protocols are further divided to consider patients who have had prior extensive radiotherapy and, therefore, cannot be treated with myelosuppressive agents. Based on objective response criteria, 5 FU, Cytoxan, Estracyt, and perhaps DTIC have proven effective and have increased survival time. Additional studies are in progress to treat clinically stable Stage D patients, and newly diagnosed Stage D patients with combinations of hormonal

chemotherapy (Johnson, et al., 1977; Scott, et al., 1976; Schmidt, et al., 1976; Johnson et al., 1976; Gibbons, et al., 1976; and Murphy, et al., 1977).

Animal models have been developed to test and predict the activity of chemotherapeutic agents or other modalities of treatment. Emphasis has recently shifted from measuring DNA as a marker of treatment response to employing the R3327 tumor model which is characterized by a battery of enzymes and morphological criteria (Coffey, et al., 1977). Short-term organ cultures of rat and human prostatic tissues are being used as a means of testing drugs potentially useful in prostatic cancer (Kadohama, et al., 1977). Optimal conditions have been established for growing such tissues, and the procedure for measuring 5 α-reductase miniaturized. Various drugs are being evaluated by their effect on this enzyme system. Drugs used in the chemotherapy trials have shown activity in either one or both of these model systems.

Emphasis and Projections

Several investigators have been successful in their efforts to grow prostate tissue in vitro either in monolayer or in direct suspension. This is itself an achievement, but a major problem still confronting these investigators is the positive identification of epithelial cells by other than morphological criteria which are considered unreliable. Investigators are applying enzyme measurements, 5 α-reductase, arginase, and other parameters such as, zinc uptake and RIA for acid phosphatase in order to obtain a positive marker for epithelial cells. This effort is necessary in order to learn more about the biologic natural history of prostatic cancer, and is a necessary adjunct to viral studies.

Several case-control epidemiological studies are in progress which are examining socioeconomic, dietary, sexual, and other factors in high- and low-risk groups in search of possible causative factors in the development of prostatic cancer.

It has been demonstrated that human tumors can be labeled efficiently with ^{111}Indium-oxine chelate, which can subsequently be released by cytotoxic lymphoid cells. Both natural and induced cytotoxicity can be demonstrated utilizing this isotope release method, and the assay can be utilized in long-term cytotoxic assays in the study of human tumor immunology.

Transrectal ultrasonic prostatic scans have been performed in patients and found to be helpful in the evaluation of patients with negative needle biopsies that warrant either repeat studies or open biopsy. The results to date indicate that this noninvasive technique is more accurate than rectal examination for evaluating the extent of local disease. Further studies are in progress to correlate the ultrasonic findings with the surgical stage of the disease.

Additional studies which remain to be undertaken or expanded include: measurement of trace metals in prostatic cancer, study of genetic factors in BPH and prostatic cancer, evaluation of intrinsic and extrinsic factors and conditions which determine the malignant character of prostatic cancer,

characterization of hormone receptors in normal, BPH and cancerous prostatic tissue, measurement of serum prolactin levels in prostatic cancer, characterization of biological markers of prostatic epithelial cells, evaluation of the diagnostic usefulness of bone marrow acid phosphatase in patients with prostatic cancer, study of the surface of normal and malignant prostatic cells, study of immunoregulation of prostatic cancer, investigation of lymph-node spread in prostatic carcinoma, use of combination cytotoxic chemotherapy of Stage D cancer of the prostate, use of adjuvant chemotherapy for carcinoma of the prostate in patients with positive pelvic or paraaortic lymph nodes, evaluation of elective radiotherapy of axial skeleton in patients with Stage C carcinoma of the prostate, and evaluation of treatment modalities and study of factors that potentiate tumors in animal models of prostatic adenocarcinoma.

Other Program Activities

The National Prostatic Cancer Project sponsored a Workshop on Prostatic Cancer, June 28 to 29, 1976, in Bethesda, Maryland. Another workshop evaluated the usefulness of *in vivo* and *in vitro* systems in prostatic cancer, March 14, 1977, in Buffalo, New York.

Three special publications were sponsored: Seminars in Oncology, Vol. III, 1976, Carcinoma of the Prostate, Editor, Gerald P. Murphy; Cancer Chemotherapy Reports, Vol. 61, 1977, National Prostatic Cancer Project Workshop on Prostatic Cancer, in press; and Oncology, Vol. 2, 1977, Workshop to Evaluate the Usefulness of *In Vivo* and *In Vitro* Systems in Prostatic Cancer In press.

Two special activities were sponsored: development of a home study course for the continuing education program of the American Urological Association on carcinoma of the prostate, Gerald P. Murphy; and American Urological Association tape seminar for urologists on non-hormonal treatment for carcinoma of the prostate, Gerald P. Murphy.

NATIONAL PROSTATIC CANCER PROJECT

(Dollars in Thousands)

NONCOMPETING		COMPETING		TOTAL	
NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT
27	$2,121	15	$1,459	42	$3,580

REFERENCES

Choe, B. K., et al., Immunochemical Studies of Prostatic Acid Phosphatase. Cancer Chemother. Rep. In press, 1977. (CA 18748 and CA 16426)

Chu, T. M., et al., Enzyme Markers in Human Prostatic Carcinoma. Cancer Chemother. Rep. In press, 1977. (CA 15126)

Chu, T. M., et al., Immunochemical Detection of Serum Prostatic Acid Phosphatase Methodology and Clinical Evaluation. Cancer Chemother. Rep. In press, 1977. (CA 15126)

Gibbons, R. P., et al., Prostatic Carcinoma. Relationship Between Primary Tumor, Histologic Grade, and Response to Chemotherapy. Urology 8:222-226, 1976. (CA 15407, 15421, 15107, 21438, 15108, 15284, and 14716)

Jackson, M. S., Characterization of Prostatic Carcinoma Among Blacks: A Continuation Report. Cancer Chemother. Rep. In press, 1977. (CA 15448)

Johnson, D. E., et al., Clinical Significance of Serum Acid Phosphatase Levels in Advanced Prostatic Carcinoma. Urology 8:123-126, 1976. (CA 15407, 15421, 15107, 21438, 15284, and 14716)

Johnson, D. E., et al., National Randomized Study of Chemotherapeutic Agents in Advanced Prostatic Carcinoma: A Progress Report. Cancer Chemother. Rep. In press, 1977. (CA 15107, 15407, 15421, 21438, 15108, 15284, and 14716)

Kadohama, N., et al., 5α-Reductase as a Target Enzyme for Anti-Prostatic Drugs in Organ Culture. Oncology In press, 1977. (CA 15436)

Murphy, G. P., et al., A Comparison of Estracyt and Streptozotocin in Patients with Advanced Prostatic Carcinoma Who Have Had Extensive Irradiation. J. Urol. In press, 1977. (CA 15407, 15421, 15107, 21438, 15108, 15284, 14716, and 15126)

Schmidt, J. D., et al., Chemotherapy of Advanced Prostatic Cancer. Evaluation of Response Parameters. Urology 7:602-608, 1976. (CA 15407, 15421, 15107, 21438, 15108, 15284, and 14716)

Scott, W. W., et al., The Continued Evaluation of the Effects of Chemotherapy in Patients with Advanced Carcinoma of the Prostate. J. Urol. 116: 211-213, 1976. (CA 15407, 15421, 15107, 21438, 15108, 15284, and 14716)

CLINICAL ONCOLOGY PROGRAM

Description

The Clinical Oncology Program is a consolidation of related research in the broad area of preclinical and clinical treatment. The program embraces large multidisciplinary program projects in cancer centers and academic institutions, as well as smaller, single-investigator grants with well defined projects of limited scope. Although the major thrust of the research effort is treatment, projects dealing with related topics and problems are supported. Examples of these related activities are: (1) research in supportive care methods (blood component therapy, protected environment, control of infectious disease complications, diet and nutrition support, psychosocial therapy, etc.); (2) nonbiological models (computer models) to determine drug dose and prevention of toxicity; (3) new methods to more effectively deliver currently available drugs; (4) diagnosis and control of cancer-related disorders (paraneoplastic diseases); and (5) organ transplantation (bone marrow, stem cells, etc.).

In addition to clinical activities in the broad area of cancer therapy (clinical trials), specific disease entities are investigated in total. Examples of this type of approach are projects in leukemia, trophoblastic disease, anemias associated with malignancy, nervous system tumors, lymphomas, lung cancer, breast cancer, myeloma, etc. Other programs integrate the study of these disease classes with basic research components with long-range goals.

Most of the program grants support a critical mass of investigators who possess a variety of expertise and who often serve as a resource to other programs in the National Cancer Institute. Examples are primary drug testing and pilot clinical trials that feed into large clinical cooperative group protocols. In addition to the development of sophisticated new treatment methods, these investigators also serve as a resource for services that require advanced and complicated support methods.

Accomplishments

The dynamics of the circulating stem cell suggest that quantitative changes may reflect a variety of regulatory mechanisms concerned with self-renewal, proliferation, and maturation of these cells and the more mature cells of the hematopoietic system. Since the major toxicity of current chemotherapeutic regimens is myelosuppression, an understanding of stem cell kinetics is important. The ability to obtain and preserve these stem cells in sufficient quantities for reconstitution may provide the opportunity for escalating the dose of effective chemotherapeutic agents. If successful, this could lead to a greater antitumor response, increased duration of remission, and ultimately prolonged survival. The isolation of growth factors is also important. Two significant findings in this area have been reported by grantees of the program.

Yankee and his co-workers (Yankee, 1976) have been studying the stem cell in the circulating blood of normal volunteers and patients undergoing cancer chemotherapy. They have found a significant increase in circulating stem cells at certain periods following doses of various anticancer drugs. Using the Haemonetics apparatus, they have been able to collect large quantities of these stem cells and store them for long periods with a high rate of recovery. This accomplishment is significant because patients who would otherwise require reduction in drug dose may now undergo more intensive therapy with reconstitution of their marrow elements with these stored stem cells.

The second significant advance in this area of basic investigation was the isolation of the colony-stimulating factor (CSF) by Shadduck (Shadduck, 1976). This investigator has been able through a process of ultracentrifugation, ethanol extraction, DEAE cellulose, con-a-sepharose, and sephadex G150 column to isolate the molecule. The isolation and purification of this molecule has great significance for further investigation into bone marrow physiology, as well as white blood cell disorders (leukemias).

One of the traditional methods for determining drug dose and scheduling is the peripheral white blood cell count. Depending upon the level, drug dose is maintained, reduced, or escalated. This single value (albeit predictive) is not an absolute measure of the true bone marrow status, i.e., the bone marrow reserve. Brubaker (Brubaker, et al, 1976) has developed a new test called the "coil test" which is simple, fast, and will measure the reserves of white cells maintained in the bone marrow and marginal vascular pool. This method does not use drugs and it is more reliable. The method is now ready for wide-scale clinical testing to evaluate its utility for monitoring bone marrow toxicity secondary to cancer treatment, as well as other nonmalignant disease processes.

Using a new data management system called "CLINFO" (developed through another NIH grant), Morrison (Morrison, et al, 1976) has collected data on over 400 cancer patients. These data concern the serum clearance of methotrexate while patients received very high doses of the drug. This method increases its therapeutic index and is followed by "citrovorum rescue" to prevent toxicity. Patients who have high or low clearance rates of methotrexate can be quickly identified and appropriate changes made in either the dose of methotrexate or the time frame and dose of the citrovorum rescue. To date, ten percent of these patients have been spared severe toxicity by the identification of low clearance.

Opportunistic infections in immunosuppressed and neutropenic patients continue to be a major cause of morbidity and mortality in cancer patients. Severe infections are caused by fungal and by viral agents, both of which require rapid diagnosis and therapy. Gunasekaran (Gunasekaran, 1976) has been working with certain opportunistic fungal infections in an attempt to develop an early diagnostic method. Traditional culture methods are long and tedious and often inaccurate. The clinician is, therefore, unable to quickly diagnose the disease and implement therapy. Using gas-liquid-

chromatography, this investigator has been able to identify a variety of Candida species rapidly, thus making the old culture method obsolete.

Freeman (Freeman, in press) has been studying the effect of polyinosinic polycytidylic acid (Poly I:C). In a randomized study, he has treated 22 patients with Herpes Zoster infection. Although still inconclusive, the results suggest that this drug will increase the patient's ability to resist this infection.

Intrathecal chemotherapy remains the standard for the treatment and prevention of meningeal leukemia. Bleyer (Bleyer, 1976) has shown that the traditional method of determining drug dosage, based on the patient's body surface, is less effective than giving the patient a constant dose regimen. In the same study, this investigator also demonstrated that radiation enhances the clearance of methotrexate from the cerebrospinal fluid and is a reversible phenomenon.

Emphasis and Projections

The Clinical Oncology Program continues to support research that will advance knowledge in basic and applied methods of cancer treatment research. Projects that involve both single- and combined-modality modes of therapy will be encouraged. Research in the surgical oncologic area will be stressed.

There is a growing body of knowledge in the primary and secondary prevention of neoplastic disease ready to be brought to clinical testing. Identified high-risk populations and patients who have premalignant lesions will be studied to determine the effectiveness of new agents that reverse these conditions. This type of research will be encouraged.

The effect of metabolism as it relates to nutrition and cancer therapy is still of high relevance and priority to the program.

CLINICAL ONCOLOGY PROGRAM

(Dollars in Thousands)

NONCOMPETING		COMPETING		TOTAL	
NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT
77	$20,922	30	$4,690	107	$25,612

REFERENCES

Bleyer, W. A., Savitch, J., and Holcenberg, J. S., An Improved Dosage Regimen for Intrathecal Chemotherapy. Clin. Pharm. Exp. Therap. 19: 103, 1976. (Abstract) (CA 16525)

Brubaker, L. H., Johnson, C. A., Barnes, A., Jr., Rosenstein, D. L., and Hewett, J. E., A New Test for Neutrophil Marrow Reserves and Intravascular Survival. J. Lab. Clin. Med. 87:1075, 1976. (CA 11929)

Freeman, A. I., Al-Bussam, N., O'Malley, J. A., Stutzman, L., Bjornsson, S., and Carter, W. A., Pharmacologic Effects of Polyinosinic Polycytidylic Acid in Man. J. Med. Virol. In press. (CA 18533)

Gunasekaran, M. and Hughes, W. T., Differentiation of Candida Species by Gas-Liquid-Chromatography. Abstract accepted for 2nd International Mycological Congress to be held August 1977, Tampa, Florida. (CA 20917)

Lincoln, T. L., Isacoff, W. H., Groner, G. F., Willis, K. L., and Morrison, P. F., Analysis of High Dose Methotrexate Clinical Trial Data Using CLINFO. Environmental Health: Quantitative Methods, Ed. Alice A. Whittemore, SIAM, Philadelphia, 1976, 197. (CA 19663)

Richman, C. M., Weiner, R. S., and Yankee, R. A., Increase in Circulating Stem Cells Following Chemotherapy in Man. Blood 47:1031-1039, 1976. (CA 18412)

Shadduck, R. K., Leukocyte Colony-Stimulating Factor and Inhibitor Activity. J. Lab. Clin. Med. 87(6): 1041-9, 1976. (CA 15237)

CANCER DIAGNOSTIC RESEARCH AND PREVENTION PROGRAM

Description

The Diagnostic Research and Prevention Program is primarily involved in research programs and projects related to prevention, early detection and diagnosis of cancer, and the application of such knowledge to the population at risk. In these areas, the program is concerned with research directed toward the improvement of currently existing techniques as well as the development of new procedures and modalities. To accomplish the major program objectives, the following areas are stressed: (1) promoting prevention-oriented projects such as the development of new sensitive and simple-to-use testing procedures for identifying populations at high risk to cancer through detection of predisposing factors and those associated with environmental, occupational, behavioral, and life style habits which contribute to the exposure of agents hazardous to human health; (2) evaluation and improvement of known diagnostic procedures by application to selected subgroups of the population; and (3) utilization of current leads in the field of biological science, instrumentation, and computer technology to develop new diagnostic procedures. Of particular importance in this area is the development of noninvasive diagnostic methods.

The program maintains a continuing survey and evaluation of the state of knowledge regarding populations known to be at high risk of cancer. This necessitates a close liaison with other programs of the National Cancer Institute and other Federal programs concerned with identifying potential carcinogenic agents and monitoring individuals and groups known to be at risk of exposure and ultimately at risk of developing a malignant disease. The program follows closely the developments and accomplishments in advancing the prevention, detection, and diagnosis of human cancer and is concerned with identifying special needs and opportunities where increased or specialized manpower facilities and efforts might be productive. As new opportunities for advancement are identified in the cancer prevention and diagnosis area, the program will be responsible for developing activities in these areas and for providing continuing technical and administrative support of such new or special programs once they are developed.

Research Accomplishments

Congenital dysplasia of bones, which poses a diagnostic challenge, disturbs one of the underlying factors which controls the growth and shaping of bones. The disturbance is related to the importance of that governing factor in the growth and shape of the bone. Lodwick and Farrell (1976) and McFarland and Oestreich (1976) have set up a data base of 170 anteroposterior knee radiographs which has been analyzed utilizing a small computer to extract shape measurement of the lateral and medial bone contours. The data base consists of 73 normal, 27 achondroplasia, 56 metaphyseal chondrodysplasia, and 15 hypochondroplasia cases. Computer diagnosis of normal versus abnormal scored an overall accuracy of 82 percent with 94 percent accuracy between normal and achondroplasia, 80 percent accuracy between normal and metaphyseal chondrodysplasia, and 73 percent overall accuracy in a three-way analysis of normal versus achondroplasia

versus metaphyseal chondrodysplasia. These results have been obtained using only a small segment of each medial and lateral contour. Second order polynomial coefficients were extracted from these contours utilizing the least-squared-error fitted curve.

During the past year Reinke, Silverman, Rosen, and Hellan (1976) have completed the design, development, and early field testing of a matrix video-densitometer as a screening test for the early detection of pulmonary carcinoma. This electronic sampling device generates a matrix of points, 32 points on a side, thus giving a rectangle of 1,024 points. Each point functions as an independent video-densitometer cursor with 10 bits of gray level information. The matrix can be expanded to cover the majority of the television field or contracted down to approximately 6 percent of the video field for high resolution studies. The device has internal buffering so that rapid data sampling may be obtained from the recorded image of the patient's lung and passed through for computer storage and later processing. Initial laboratory testing of this matrix densitometer and its interface with the computer is under way. Clinical testing of human subjects has been carried out under the direction of Dr. Robert Reinke. Clinical testing will determine if the device is successful as an early method for detecting primary pulmonary neoplasm. Early detection may result in an improved surgical cure rate.

Van Herle (1976) has shown that measuring serum thyroglobulin (HTg) levels is useful in diagnosing thyroid carcinoma. It is also a simple and inexpensive way to evaluate the presence of metastatic disease in cases with established differentiated thyroid malignancies. However, in patients with suspected thyroid malignancy, the HTg test is not specific enough to distinguish between benign lesions which have elevated HTg levels, from thyroid carcinoma (Van Herle, Chopra, Hershman, and Hornabrook, 1976). An investigation of the suppressive effect of L-thyroxine on HTg release in patients with thyroid nodules may reveal the reason for their elevated HTg serum levels (Shimaoka, Van Herle and Dindogru, 1976).

Excellent results have been obtained from electron paramagnetic resonance (EPR) studies on the detection of breast cancer in a prebiopsy program (Horn, Friesen, Lan, Hedrick, Stephens, and Zimbrick, 1977). Ceruloplasmin serum levels as measured by EPR indicate that patients with benign breast lesions (such as fibrocystic disease) have levels which are higher than those obtained from normal volunteers of similar ages. Patients with cancerous lesions had significantly higher levels than normal women or women with benign lesions. Levels in the three groups follow: normal volunteers average 3.46 ± 0.66 ; patients with benign lesions average 4.46 ± 0.71; patients with breast cancerous lesions average 7.24 ± 1.95. Further data are being obtained to evaluate the hypothesis that serum ceruloplasmin, as measured by EPR, is a marker for breast cancer at the prebiopsy stage (Horn, Friesen, Lan, Hedrick, Stephens, and Zimbrick).

The development of new radioactive scanning compounds has been directed towards agents which localize specifically in malignant soft tissues or in organs of interest. The synthesis of ^{11}C-Valine would provide an agent with a high degree of localization in the pancreas. Localization of ^{11}C-Valine is almost

twice that of ^{75}Se-selenomethionine, the agent currently in use. Thus, ^{11}C-Valine should provide improved visualization of the pancreas and detection of smaller lesions with greatly reduced radiation exposures.

Selenite has been shown to localize in a broad spectrum of soft tissue and osseous tumors. Substitution of ^{73}Se for ^{75}Se will permit larger amounts of activity to be administered, and will result in a major reduction in radiation dose, more precise localization, and resolution of smaller concentrations of selenium in malignant tissue. This should enable earlier cancer detection (McDonald, Gelbard, Clark, Christie, and Lauglin, 1976).

Experiments performed in an animal model that bears a transplanted carcinoembryonic antigen (CEA) producing human colonic carcinoma demonstrate that radioantibodies made against CEA can be used to selectively localize the tumor by total-body photoscanning. By producing a purified anti-CEA, the localization ratios have been greatly improved. The CEA and nonspecific cross reactive antibody (NCA) localized equivalently in hamsters bearing the GW-39 colonic carcinoma. These studies form a basis for clinical trials (Primus, MacDonald, Goldenberg, and Hansen, 1977).

Other Program Activities

The program supported a conference of the Panel on Diagnostic Application of Radioisotopes in Haematology of the International Committee for Standardization in Haematology. The panel's newly completed document on platelet survival studies has been accepted for publication in the journal Blood. The document presents new recommendations for standardizing the measurement of platelet survival. Three further publications are anticipated.

The program also supported the International Cancer Detection and Prevention Symposium held April 26 - May 1, 1977 in New York City. The purpose of the symposium was to facilitate widespread propagation of new knowledge, and promote closer links with other national and international organizations engaged in cancer prevention and control. Following the pattern set by the first two symposia, the proceedings will be published and distributed early next year.

Emphasis and Projections

Evidence continues to mount that human cancer has its genesis in our environment. The environment, which contains the cancer-causing agents, may encompass a broad spectrum of exposures to contaminants, additives, physical agents and stresses, or even natural sources of materials such as mycotoxins, plant toxins, and those of geologic origin. During the last 50 years, thousands of new chemical substances have been introduced into the environment. A large number of these compounds have been demonstrated to be carcinogenic in rodents and about 17 of them are known to be carcinogenic in man. The carcinogenic effects of some of these substances may be enhanced by cigarette smoking, alcohol consumption, or diet. In view of the increased risk for certain cancers related to the work place and its environment, emphasis is being given to the development of occupational cancer-prevention research.

DIAGNOSTIC RESEARCH AND PREVENTION

(Dollars in Thousands)

NONCOMPETING		COMPETING		TOTAL	
NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT
21	$1,613	12	$1,220	33	$2,833

REFERENCES

Horn, R. A., Friesen, E. J., Lan, C. F., Hedrick, W. R., Stephens, R., and Zimbrick, J. D., Qualitative and Quantitative Changes in Ceruloplasmin in Blood from Normal Human Subjects and Cancer Patients. Accepted by Cancer Res., 1977. (CA 14335)

Lodwick, G. S. and Farrel, C., Criteria for Radiologic Distinction of Malignant from Benign Tumors of Bone. Weekly Radiology Science Update 34, 1977. (CA 06263)

McDonald, J. M., Gelbard, A. S., Clarke, L. P., Christie, T. R., and Laughlin, J. S., Imaging of Tumors Involving Bone with 13M-Glutamic Acid. Radiology 120:623-662. (CA 18153)

McFarland, W. D. and Oestereich, A. E., A Method for Computer-Assisted Description of Bone Shape in the Automated Diagnosis of Dysplasias, Annual Conference on Engineering in Medicine and Biology, New Orleans, La., Sept. 22, 1976. (CA 06263)

Primus, F. J., MacDonald, R., Goldenberg, D. M., and Hansen, J. J., Localization of GW-39 Human Tumors in Hamster by Affinity-purified Antibody to Carcinoembryonic Antigen. Cancer Res., 1977. (CA 17742)

Reinke, R. R., Silverman, N. R., Rosen, L., and Hellan, D. H., Pulmonary Videodensitometry in the Diagnosis of Carcinoma of the Lung. Radiology 118:521-525, 1976. (CA 16383)

Shimaoka, K., Van Herle, A. J., and Dindogru, A., Thyrotoxicosis Secondary to Involvement of the Thyroid with Malignant Lymphoma. J. Clin. Endocrinol. Metab. 43:64, 1976. (CA 13447)

Van Herle, A. J., Serum Thyroglobulin Assays, 1976. In: Conference on Radiation-associated Thyroid Cancer. Leslie J. DeGroot (ed), Chicago. (CA 13447)

Van Herle, A., Chopra, I. J., Hershman, J. M., and Hornabrook, R. W., Serum Thyroglobulin In Inhabitants of an Endemic Goiter Region of New Guinea. J. Clin. Endocrinol. Metab. 43:512, 1976. (CA 13447)

DRUG DEVELOPMENT AND PHARMACOLOGY PROGRAM

Description

The Drug Development and Pharmacology Program is concerned with the development and evaluation of chemotherapeutic agents for more effective treatment of malignant disease. This involves specific or selective destruction of neoplastic cells present in the host without affecting the viability and function of normal cells. The primary objective of the Program is to provide scientific direction and encouragement to investigators in diverse scientific disciplines to pursue research in the chemical, pharmacological, and cytological aspects of abnormal and normal growth processes. Within this broad framework, preclinical and clinical research is planned and implemented through various stages of drug development.

The search for effective agents in the control of cancer begins with the synthesis of potential chemical agents prepared by synthetic chemical processes or by their isolation from microbial, plant, or animal sources. Subsequent stages include preclinical evaluation of antitumor activity in experimental therapeutic test systems, preclinical toxicologic and pharmacologic evaluation, studies in cell kinetics, and the establishment of criteria for selection of safe dosage regimens. Investigations are conducted on the physiological disposition, metabolism, and biotransformation of selected drugs which might lead to an understanding of the mechanisms of drug action and growth inhibition. Clinical pharmacology includes investigations on pharmacologic evaluation of potential clinical drugs during controlled clinical trials. The object is to correlate the preclinical data with clinical findings for a better understanding of the mechanisms of drug actions and selection of more effective treatment regimens. Each of these component areas--synthesis, experimental therapeutics, pharmacology, and biochemical mechanisms of action--is an integral part of the total drug development program. These activities encompass the integrated research efforts of the chemist, biochemist, pharmacologist, and the chemotherapist.

Clinical findings of the past decade have demonstrated that the use of chemical agents, mostly in various combinations in the treatment of disseminated cancer, has resulted in a significant degree of palliation or rate of cure of a variety of neoplasms, particularly those of the rapidly proliferating type. Progress has been less spectacular on slow-growing tumors, e.g., carcinoma of the lung and colon. Considerable promise of progress in this area is anticipated through the use of mixed modality therapy which includes chemotherapeutic agents with surgery, radiation, or immunotherapy. All research activities within the Drug Development and Pharmacology Program relate to the preclinical Treatment Research thrust.

Accomplishments

Total Synthesis of Vernolepin and Vernomenin: Vernolepin and vernomenin, two rare, naturally occurring dilactone compounds, have now been synthesized

in the laboratory (Danishefsky, et al., 1976) and (Grieco, et al., 1976). Vernolepin, the major active principal, exhibits inhibitory activity against the Walker intramuscular carcinosarcoma 256 in rats at 12 mg/kg. Recent data in structure activity correlation experiments indicate that the presence of 2-methylene-r-butyrolactone moiety is essential for in vivo antitumor activity. Vernolepin and vernomenin will now undergo further preclinical evaluations. Studies are also under way (Danishefsky, et al., 1976) to improve on the total synthesis of vernolepin and vernomenin with regio-specificity.

Liposomal Potentiation of Antitumor Activity: The delivery and distribution of proteins and drugs in vivo are altered when drug molecules are entrapped in phospholipid liposomes. Lipid vesicles can enter cells, in vitro and in vivo, without apparent deleterious effect. Pharmacologically active substances, including those which cannot ordinarily enter cells, can be incorporated into these vesicles. These may then be specifically directed to the cell surface or other regions of the cell, depending on the type of vesicle, to exert a desired effect and thus modify the cell's behavior. This innovative technology might be of considerable biological and clinical importance for the delivery of cancer drugs in man.

During the past year, evidence was reported (Rutman, et al., 1976) of liposomal activation of two highly reactive and unstable alkylating agents, BCNU and mechlorethamine, against two mouse tumors, Ehrlich ascites and L1210 leukemia. The effects of mechlorethamine on the Ehrlich ascites tumor were increased when either a single- or a multiple-dose protocol was used, and the effects of the multiple-dose regimen were quite significant. Similar results with the Ehrlich ascites tumor were also obtained in the single-dose experiment with BCNU. It is conceivable that the drugs could dissolve in the bilipid membrane layers and thus bypass the permeability barrier of the cell membrane because liposomes can fuse with cell membranes as well as enter cells. Another possibility is that the liposome vesicle facilitates drug attack on membrane-bound biosynthetic complexes. There is an important need to establish the mode of carriage by the liposomes of drugs such as mechlorethamine and BCNU.

Distribution of Liposome-Entrapped Methotrexate (MTX): It has been demonstrated that entrapment of methotrexate with positively charged liposomes greatly altered the subsequent distribution of MTX in the cynomolgous monkey after a single injection (Bourke, et al., 1976). Observations from this study indicate that liposome-entrapped MTX should decrease toxic gastrointestinal symptoms often found during MTX treatment as well as reduce renal impairment. Sonically disrupted, positive liposomes are preferable since they are able to maintain high plasma levels of MTX with the MTX showing little metabolic breakdown or toxicity. Liposomes thus provide a useful basic drug carrier that is not readily taken up or excreted from the body. Although liposomes appear to be of little use in directing entrapped material into the intact central nervous system, they might be of use in cases where there is local breakdown of the blood-brain barrier such as in tumors. Specific uptake of unmodified liposomes into tumor tissue has not yet been observed. However, treatment of tumor-bearing

mice with liposome-entrapped actinomycin D led to a 50 percent increase in survival time compared with the same concentration of free actinomycin D.

The important prerequisite for successful attack on cancer cells with antitumor agents is specificity. It appears that by directing the drugs with liposomes, the liposome carrier might be able to recognize and to penetrate the specific area of action. Following their liberation from liposomes, drugs could then proceed for a finer target recognition at the molecular level resulting in enhanced therapeutic effect.

Polyamines as Tumor Markers: Elevated levels of the polyamines-putrescine, spermidine, and spermine, have been found in serum, urine, and cerebrospinal fluid of patients with a wide variety of neoplasms. These polyamines are involved in cell growth, with possible significance in the regulation of RNA-dependent protein synthesis. The most marked synthesis and accumulation of polyamines occur in rapidly growing tissues. In one study, polyamine levels were directly related to disease activity and tumor burden (Russell, et al., 1976). In patients with multiple myeloma, putrescine levels were significantly correlated with clinical disease activity as well as the in vitro labeling index of marrow plasma cells. Spermidine values reflected tumor cell burden. Serial studies in 56 patients indicated that greater than twofold rise in urinary spermidine during treatment was highly correlated with cell kill and subsequent clinical response. Serum polyamine levels in 17 patients were found to be comparable to urinary values. These data indicate that polyamine determinations might be potentially useful as clinical indicators of tumor cell mass and growth fraction. Increase in spermidine during treatment is considered to be an excellent marker of tumor cell kill.

In another study with polyamines, (Marton, et al., 1976) cerebrospinal fluid (CSF) polyamine concentrations were assayed in patients with and without central nervous system tumors. Statistically valid elevations were found in the CSF polyamine concentrations of patients with untreated malignant central nervous system tumors when compared with those concentrations observed in the CSF of patients without neoplasia. Patients undergoing successful tumor therapy for malignant central nervous system tumors showed CSF polyamine concentrations that closely approximated the concentrations found in CSF of patients without tumor. It appears that significant increases, particularly in putrescine concentrations precede a decline in clinical status due to tumor regrowth.

In exploring the value and significance of polyamine determinations in the diagnosis of various types of neoplasia and in the evaluation of the efficacy of tumor therapy, it is important to realize that primary diagnosis of brain tumors in particular may be difficult, and that conventional diagnostic procedures are not devoid of risk. A search for diagnostic markers in cerebrospinal fluid has been under way for a number of years with only minimal success to date. Studies are continuing in order to verify these potentially useful findings. A marker for judging the effectiveness of therapy in an extremely short survival expectancy disease would be critical to the eventual success of brain tumor therapy.

Adriamycin-Polynucleotide Complexes and DNase: In an effort to reduce the toxicity of the anticancer agent adriamycin, studies have been made on the use of adriamycin-DNA complex as an approach to improve the therapeutic effectiveness of adriamycin for use in cancer chemotherapy (Tsou, et al., 1976). Data on the interactions of adriamycin with single-strand polydeoxyribonucleotides, double-strand DNA, and double-strand ribodeoxyribopolynucleotide hybrids showed that the inherent variable in such a complex lies in the composition of the polynucleotides. The beneficial effect of using such a complex over using the drug alone depends on the fact that the complex is less toxic than the drug. This research, relating to the enzymatic basis of the approach, concluded that adriamycin effectively intercalated ribodeoxyribopolynucleotide hybrid duplexes. The adriamycin-hybrid complex should be a compound of low toxicity and the adriamycin could be released only by the action of DNase II, a nuclease that has been found to associate with RNA-dependent DNA polymerase related to viral infection. The significance of this research lies in the importance of improving existing chemotherapeutic agents through understanding their mode of action in an effort to increase their therapeutic efficacy with reduction in toxicity to the normal tissues of the patient.

Methotrexate and 5-Fluorouracil Drug Interaction: A controversy has arisen in connection with the use of methotrexate, one of the most frequently used anticancer drugs. If preliminary findings are substantiated, many commonly used regimens for combination chemotherapy of tumors may have to be revised. The preliminary research indicates that methotrexate interferes with the activity of 5-fluorodeoxyuridine given at the same time. The compound 5-fluorouracil is believed to have the same mechanism of action as 5-fluorodeoxyuridine (FldUrd) (Santi, et al., 1977. In press.).

FldUrd and 5-fluorouracil are metabolized to FldUrd monophosphate, the active form of the drug. The monophosphate inhibits the activity of thymidylate synthetase thus inhibiting the synthesis of DNA and impairing the replication of tumor cells. Methotrexate and FldUrd or 5-fluorouracil have been believed to act in a complementary way. Studies now indicate, however, that methotrexate could interfere with the activity of 5-fluorouracil and FldUrd by inhibiting biosynthesis of the co-factor involved, 5, 10-methylenetetrahydrofolic acid. The studies may result in a more effective administration schedule of the drugs. The activities of the drugs can be complementary if they are given in the proper sequence. If methotrexate is given before FldUrd, or simultaneously, its irreversible effects on synthesis of the co-factor inhibit the action of FldUrd. The effects of the two drugs might remain complementary if they are not given until after the effects of FldUrd have reached their peak.

Pharmacology of Chlorozotocin: Extensive studies on structure-activity relation of nitrosourea derivatives and their pharmacologic properties led to the synthesis of a unique, water-soluble nitrosourea compound, chlorozotocin. This compound possesses significant antitumor activity against leukemia test systems.

Chlorozotocin produces only a minor degree of inhibition of mouse and human bone marrow DNA synthesis compared to Bis-chloroethyl-nitrosourea (BCNU). This bone marrow sparing feature of chlorozotocin is relative and if the drug is administered at lethal dose levels in mice, myelosuppression is observed. These observations have now been extended to normal human bone marrow (Schein, et al., 1976). The potential importance of these studies is the identification of a new and active nitrosourea antitumor agent with modified bone marrow toxicity. If aminoglucose modification of nitrosourea bone marrow toxicity can be confirmed in man without significant loss of antitumor activity, the use of such a compound could facilitate treatment of cancer patients who have preexisting abnormal bone marrow function. It would also allow for more effective use of a nitrosourea agent in combination with anticancer agents possessing more potent myelosuppressive properties.

Emphases and Projections

Nutritional Control: Manipulation of nutritional factors, either by supplementation or by creation of a deficiency, has already been found to be effective in the therapy of a few specific neoplasms. Particular examples are the Walker 256 carcinoma, which is sensitive to folate deficiency but not to methotrexate administration; certain lymphoblastic leukemias which are relatively sensitive to asparagine deficiency; and some lymphomas which are sensitive to riboflavin deficiency. However, the area is as yet relatively unexplored because of difficulties in obtaining nutritional depletion and due to lack of knowledge of nutritional requirements by which specific tumors might differ from the requirements of their host.

Deficiencies of certain vitamins, in particular folic acid, vitamin B_{12}, pyrodoxine, and riboflavin, have been identified as potential emphasis areas that may be worth further exploitation for more selective antineoplastic effects. Methods to deplete body stores of certain nonessential amino acids, in particular asparagine, glutamine, serine, glycine, and cysteine are also worthy of further investigation. Other promising approaches for improvement of the results of cancer chemotherapy are the use of specific nutrients to enhance the metabolite to "rescue" normal cells but not tumor cells, and finally the use of a nutrient or nutrients to stimulate growth so as to obtain an increased sensitivity to inhibitors of DNA synthesis.

Selective Toxicity: An important consideration in the design of cancer chemotherapy is the selective toxicity which, in part, depends on key differences between the amount, protein nature, and activity of potential key enzymes that play a crucial role in the growth process. A study of the mechanisms controlling gene expression in normal and neoplastic cells should reveal specific points of attack to exert control over the function and proliferation rate of cancer cells. These areas will remain the targets of intensive emphasis in future research. Nonspecific cytotoxicity is the limiting factor in the effective use of agents active in both cancer chemotherapy and the suppression of immune response, and the bone marrow is usually the dose-limiting tissue. Future emphasis will include studies on

the effects of antitumor drugs on the birth and flow of colony-forming cells (CFC) from the bone marrow to auxiliary organs such as the spleen. An analysis of CFC levels, brought about by these agents, may be useful in detecting early toxic effects on hemopoiesis. Another aspect would include analyzing the antitumor activity of cells from mice responding to injections of antitumor agents and immunopotentiators. Established clinical drugs (cyclophosphamide, nitrosoureas, vinblastine) as well as newer agents will be included in these studies.

Pharmacology of Cell Division Cycle: In the search for exploitable differences of toxicity and therapeutic responses which might exist between normal and tumor cells, a diverse range of chemical agents have been studied with respect to lethal effects, chromosome aberration induction, biochemical mode of action, and cell cycle events. The closely integrated biochemical events within the cell cycle in turn regulate certain cell functions that determine response of the cell to any given stimulus or agent. Mammalian cells, both normal and malignant, respond to chemical agents as a function of their position in the cell cycle--the nondividing cells responding in ways different from the dividing cells and the repair or recovery mechanisms possibly being response modifiers in both dividing and nondividing cell populations. The determination of the sequential biochemical events that designate the position of a cell in the cell division cycle and the influence of each of the response modifiers on the cell cycle control provide the basis for an understanding of how chemicals act on cells. This is a long-term goal to acquire knowledge which could be exploited with the result that new agents might be specifically designed to take advantage of the difference in response between the normal and malignant cells.

Other Program Activities

During the past year the Drug Development and Pharmacology Program supported a conference on the Chemistry and Biology of Nucleosides and Nucleotides. The conference brought together chemists, biochemists, and biologists of international reputation to discuss findings especially relating to those nucleotides and nucleosides which have shown anticancer activity and to define rationales for the design of unique compounds and new techniques.

The Program provided partial support for a Gordon Research Conference on Plant Cell and Tissue Culture. The Conference brought together experts in the fields of plant genetics and plant cell-culture technology. The conference is of special interest to both the Drug Development and Pharmacology Program and the Natural Products Branch of the Division of Cancer Treatment as the search for new and more effective plant antineoplastic agents, such as Maytansine, becomes increasingly relevant to cancer therapy.

Partial support was also provided for travel assistance to American chemists to attend the XXVI International Congress of Pure and Applied Chemistry held in Tokyo. Conference symposia and sessions included discussion of complex organic compounds, coordination compounds, and novel methods for

structure determinations, including new developments in the area of high-resolution molecular spectroscopy, an area important for rapid determination of structures of newly found active anticancer agents.

The Drug Development and Pharmacology Program maintains continuing liaison with related programs within the Division of Cancer Research Resources and Centers and with the other divisions in the National Cancer Institute. Interchange of information between the intramural and collaborative research segments of the Division of Cancer Treatment occurs through frequent meetings and discussions with the Decision Network Committee.

Special cognizance is maintained of program efforts supported by contracts to assure coordination of total research efforts in the Treatment thrust area. The investigator-initiated, peer reviewed research which is supported by the grants mechanism, thus remains an integral part of the total Treatment research supported by the National Cancer Institute.

DRUG DEVELOPMENT AND PHARMACOLOGY

SUMMARY BY SUB-CATEGORY
(Dollars in Thousands)

	NONCOMPETING		COMPETING		TOTAL	
	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT
SYNTHESIS & CHEMISTRY	106	$5,996	28	$1,859	134	$7,855
NATURAL PRODUCTS	28	1,589	14	947	42	2,536
SCREENING & EXPERIMENTAL THERAPEUTICS	26	1,613	4	258	30	1,871
PRECLINICAL TOXICOLOGY & PHARMACOLOGY	11	731	3	145	14	876
OTHER PRECLINICAL ASPECTS	10	679	2	155	12	834
MECHANISM OF DRUG ACTION	54	4,038	21	1,658	75	5,696
CLINICAL PHARMACOLOGY	3	177	0	0	3	177
BROAD MULTIDISCIPLINARY RESEARCH PROJECTS	0	0	0	0	0	0
PROGRAM PROJECTS	5	3,906	0	0	5	3,906
MANPOWER	44	1,700	12	106	56	1,806
TOTAL	287	$20,429	84	$5,128	371	$25,557

REFERENCES

Danishefsky, S., Kilahara, T., Schuda, P.F., and Etheredge, S.J., A Remarkable Epoxide Opening--An Expeditious Synthesis of Vernolepin and Vernomenin. J. Amer. Chem. Soc. 98:3028, 1976. (CA 12107)

Durie, B.G.M., Salmon, S., and Russell, D. H., Polyamines as Markers of Response and Disease Activity in Cancer Chemotherapy. Cancer Res. 37:214-221, 1977. (CA 14783)

Grieco, P. A., Nishizawa, M., Burke, S., and Marinovic, N., The Total Synthesis of Vernolepin and Vernomenin. J. Amer. Chem. Soc. 98:1612, 1976. (CA 13689)

Kimelberg, H. K., Tracy, T. F., Biddlecome, M., and Bourke, R.S., The Effect of Entrapment in Liposomes on the In Vivo Distribution of [3H] Methotrexate in a Primate. Cancer Res. 36:2949-2957, 1976. (CA 17516)

Marton, L. J., Heby, O., Levin, V. A., Lubich, W. P., Crafts, D.C., and Wilson, C.B., The Relationship of Polyamines in Cerebrospinal Fluid to the Presence of Central Nervous System Tumors. Cancer Res. 36:973-977, 1976. (CA 15515)

Russell, D. H., Clinical Relevance of Polyamines as Biochemical Markers of Tumor Kinetics. Clin. Chem. 23:1, 1977. (CA 14783)

Rutman, R. J., Ritter, C.A., Avadhani, N. G., and Hansel, J., Liposomal Potentiation of the Antitumor Activity of Alkylating Drugs. Cancer Treatment Reports 60:5, 1976. (CA 05295)

Santi, D. V., Martin, D. W., Lee, M., and Ullman, B., Cytotoxicity of 5-Fluoro-2'-Deoxyuridine: Requirement for Reduced Folate Co-Factors and Antagonism by Methotrexate. Science 194-310, 1976.

Schein, P. S., Panasci, L., Woolley, P. V., and Anderson, T., Pharmacology of Chlorozotocin (NSC - 178248), a New Nitrosourea Antitumor Agent. Cancer Treatment Reports 60:6, 1976. (CA 17583)

Tsou, K. C. and Yip, K. F., Effect of Deoxyribonuclease on Adriamycin Polynucleotide Complexes. Cancer Res. 36:3367-3373, 1976. (CA 07339)

RADIATION PROGRAM

Description

Since 1972 the Radiation Program has been jointly administered by a permanent Program Director for Radiation Biology and Physics and a rotating Program Director for Radiation Therapy. The latter role is customarily filled by an academic Radiation Therapist on sabbatical leave. In fiscal year 1977 this position was occupied by Dr. Ralph Scott of the University of Louisville, Louisville, Kentucky.

Radiation can both cause and cure cancer under appropriate conditions, a characteristic shared by at least some cancer chemotherapeutic agents. The purpose of the Radiation Program, therefore, is to support meritorious research whose ultimate goal is to reduce radiation-induced cancer incidence and increase radiation-induced cancer cure. At the basic mechanism level, information generated may be equally valuable to radiation carcinogenesis and radiation therapy.

In fiscal year 1977, the overall program consisted of the approximately equally funded portions: traditional research grants (primarily radiation biology), specialized Radiation Research Centers (basic and clinical), and high LET (linear energy transfer) Radiation Research Centers. The latter area was selected for emphasis at the beginning of this decade and continues its development. It includes radiations such as neutrons, protons, pions, alpha particles, and heavy ions which have generated great interest in the Radiation Therapy community in recent years.

Other areas of substantial current interest in the Radiation community and in the Radiation Program include hyperthermia, hypoxic radiation sensitizers, and the combination of radiation and hyperthermia.

Research Accomplishments

The most exciting and potentially the most promising work in radiation therapy research today is in the high LET area. Presently the NCI is supporting five programs aimed at clinical evaluation of fast neutrons, one for protons, one for pions, and one for heavy ions.

The five neutron efforts are at (1) the University of Washington, Seattle, Washington (Parker, 1976); (2) Texas A&M under the direction of M.D. Anderson Hospital (Caderao, 1976); (3) the Naval Research Laboratory under the direction of George Washington University; (4) the National Accelerator Laboratory (Fermilab) outside Chicago; and (5) the NASL-Lewis facility under the direction of the Cleveland Clinic. At the first four facilities, observations have been made on tumor responses and normal tumor tolerance on approximately 800 patients. Phase I trials on a variety of human cancers will continue concurrent with studies on normal tissue tolerances, optimum doses, and patterns of treatment application. In addition, randomized clinical trials on a number of appropriate tumor sites were begun during fiscal year 1977.

Phase I trials are also under way with protons at the Harvard Cyclotron Laboratory under the direction of the Massachusetts General Hospital, with pions at the Los Alamos Scientific Laboratory under the direction of the University of New Mexico (Kligerman, 1976), and with heavy ions at Lawrence Berkeley Laboratory under the direction of the University of California, San Francisco. In all these facilities, randomized clinical trials are still some years away.

The postulated advantages of various high LET radiations in a therapy situation may be either physical (better dose distribution), biological, or a combination of both. Although a number of biological mechanisms have been suggested, the most commonly cited has to do with the role of oxygen in cellular radiation damage. Essentially, tumors of appropriate size have a core of lowered oxygen concentration and oxygen is a radiation-sensitizing agent, but more so for photons than for high LET radiation. Thus, hypoxic tumors may be more susceptible to high LET radiations than to photons.

Two other active areas of radiation research include (A) the use of elevated temperature with radiation i.e., hyperthermia plus radiation (Henle, 1976), and (B) the use of drugs which mimic the role of oxygen in radiation therapy (hypoxic radiation sensitizers). The level of hyperthermia research has risen dramatically because of the interest stimulated by the NCI-sponsored First International Conference on Hyperthermia in April 1975. Research into "hypoxic radiation sensitizers" appears to be ready for expansion. In September 1977, NCI is sponsoring the Eighth Grey Conference on "Hypoxic Cell Sensitizers in Radiobiology and Radiotherapy" at Cambridge, England. Up until now, most research on this subject has been done outside the United States.

Emphasis and Projections

Emphasis for the next few years will continue in the very active areas of research of high LET, hyperthermia, and radiation sensitizers. This is clearly indicated by their potential significance. If the promise of high LET radiotherapy materializes, it may take ten years and $300 million to do an adequate evaluation of its full potential.

Other areas of emphasis include dynamic radiotherapy and CT scanning as it applies to treatment planning for radiotherapy. Emphasis will continue to be placed on basic radiation research with its dual potential for exploitation by means of prevention (by better understanding radiation carcinogenesis) and treatment.

Other Program Activities

The conferences will continue to stimulate and maintain researcher interest in areas ripe for exploitation. The aforementioned conference at Cambridge, England, in September 1977 is a good example. Another is the Particle Accelerators in Radiotherapy Conference (high LET radiotherapy) held in Berkeley, California, in September 1976.

Funds for radiation development have been available for a third year and have supported areas of high LET and hyperthermia research, especially as it relates to equipment development.

RADIATION PROGRAM

(Dollars in Thousands)

	NONCOMPETING NO.	NONCOMPETING AMOUNT	COMPETING NO.	COMPETING AMOUNT	TOTAL NO.	TOTAL AMOUNT
RADIATION THERAPY	104	$14,843	37	$14,079	141	$28,922
RADIATION DEVELOPMENT	12	2,700	1	1,550	13	4,250
TOTAL	116	$17,543	38	$15,629	154	$33,172

REFERENCES

Caderao, J. B., et al., Fast Neutron Radiotherapy for Locally Advanced Pelvic Cancer. Cancer 37(6):2620-9, 1976. (CA 12542)

Henle, K. J., et al., Combinations of Hyperthermia With Radiation. Radiology 121(2):451-4, 1976. (CA 11602)

Kligerman, M. M., et al., Initial Comparative Response to Peak Pions and X-Rays of Normal Skin and Underlying Tissue Surrounding Superficial Metastatic Nodules. Am. J. Roent. 126(2):261-7, 1976. (CA 16127)

Parker, R. G., et al., Early Assessment of Normal Tissue Tolerance of Fast Neutron Beam Radiation Therapy. Cancer 38(3):1118-23, 1976. (CA 12441)

CANCER CENTERS BRANCH PROGRAMS

Description

The Cancer Research Center Program activated in 1961 is designed to provide grants for the support and development of cancer complexes which engage in clinical and basic research, search for and discover ways to improve the diagnosis and treatment of cancer patients, train an effective group of physicians and investigators for the future, and hasten the upgrading of cancer care in surrounding areas. During the present decade, cancer centers have increased significantly in number and have diversified in function according to the needs, special facilities, and personnel available in differing areas of the country.

There has been increasing emphasis on centers of broad scope which can provide a comprehensive, multidisciplinary attack on cancer problems. The National Cancer Act of 1971 and the National Cancer Act Amendment of 1974 stimulated this trend by providing for the establishment of additional cancer centers. Because they serve as foci for a variety of high-priority thrusts in the attack on cancer, these centers have assumed an increasingly important and visible role in the intensified strategy of the National Cancer Program.

The Centers Program develops, directs, and administers a program of exploratory grants and core support grants for comprehensive and noncomprehensive cancer centers, evaluates the effectiveness of the Cancer Centers Program, identifies as components of center programs the cancer-related basic and clinical projects in the Biological Research and the Diagnosis and Treatment Programs, coordinates program activities with related activities in other Divisions of the Institute, reviews and presents grant applications and provides special reports as required to the National Cancer Advisory Board, and maintains liaison with the appropriate Federal and non-Federal organizations, institutions, and scientists.

Accomplishments

The guidelines for cancer centers were revised in fiscal year 1976 to include emphasis on the Cancer Centers Support (Core) Grant (CCSG). The purpose of the CCSG is to provide a means of support for those elements of a cancer center that are required for planning, development evaluation, administration, and maintenance of an active and unified cancer center in order to consolidate and focus cancer-related activities in a single administrative and programmatic structure. A CCSG can contribute to the stability of the center, to administrative and programmatic control of center activities, and to fiscal accountability and responsibility. It provides support for professional staff, centralized services and resources, and shared equipment and developmental projects. Day-to-day research and component cancer center functions are funded through other mechanisms, i.e., research project grants, education grants, contracts, and sources of support other than NIH.

Proposals for CCSG's undergo scientific and technical peer review. Effective in fiscal year 1977, the primary review for all CCSG's is carried out by the Cancer Center Support Grant Review Committee in the Review and Referral Branch of the National Cancer Institute. An important criterion is a record of cancer activities of high quality and an administrative organization that will promote and conduct a concentrated interdisciplinary attack on the cancer problem.

In fiscal year 1977, 64 CCSG's were awarded for a total of approximately $57 million. Included are two new grants, ten renewal grants, three supplemental grants, and 52 continuation grants. The total amount of CCSG support requested for competing grants in fiscal year 1977 was $50 million, with $24 million recommended by the NCAB.

The NCI has, since 1966, conducted a grant program to support the costs of exploratory studies in cancer, including planning for new cancer centers, expansions, and modification or strengthening of existing centers. These exploratory grants allow an institution to do a careful analysis of the various alternatives available to develop a cancer program consistent with local needs and organizational structures, as well as to contribute to the goals of the National Cancer Program. Exploratory grant applications are peer reviewed and a site visit is almost always made as part of the review process.

Exploratory grants are not limited to cancer center activities, but may be used for planning a number of cancer programs, facilities, and resources. The planning involves definition of goals and objectives for the cancer program to provide a base on which organization, financing, and facilities for the proposed cancer program can be developed. Feasibility studies that permit the applicant to gather data are included in the concept of planning, but may not be used to provide preliminary research data for research grant applications.

In fiscal year 1977, the Centers Program awarded 12 exploratory grants at approximately $1,200,000 in total costs. These figures include three new, two renewal, and seven continuation grants. Competing exploratory grant applications requesting $3.1 million were reviewed resulting in a recommended amount of $2.2 million.

Projections

The separation of large center grants ("umbrella") into CCSG's plus a number of clinical and/or basic program project grants and regular research project grants has been completed, and the Centers Branch now administers CCSG and exploratory grants with little direct support for research. The research that is supported is limited to developmental projects within CCSG's which will provide the center flexibility in developing new programs or strengthening existing programs to correct areas of weakness within the center. These requests for developmental "seed" funds are documented in the grant application. Developmental funds may be used for the inauguration of new projects by providing salary support for recruiting promising investigators and other professional personnel together with the appropriate personnel, equipment,

supplies, or hospital costs needed to begin their work. Such new projects must be described in detail. Investigators receiving support in this manner are required to seek funds for continuation of these developmental projects through the usual competitive funding mechanisms. CCSG funds may not be used for more than three years for any one developmental project.

Evaluation of cancer centers will continue and extensive studies are under way to develop Cancer Centers Profiles. Data collection activities are in progress and will continue. These profiles will also be used in developing long-range plans for the Cancer Centers Program.

Evaluation of the degree of comprehensiveness among cancer centers is a continuing study utilizing site visits under the aegis of the NCAB. The review of existing comprehensive cancer center programs will continue in order to document and upgrade the quality of these centers.

Other Activities

Experience since 1971 in the development of cancer centers has shown that supporting services relating to data management are essential. These services include the availability of epidemiologic, biostatistical, and computer science expertise and computer systems to each cancer center scientist and physician without regard for his research interest.

Concurrent with the development of epidemiological, statistical, and computer resources in the individual cancer centers has been the planning and early implementation of a centralized patient data system among comprehensive cancer centers. This centralized patient data system, now in feasibility testing stages, is designed to encourage the use of uniform cancer nomenclature and staging in clinical practice and research and to facilitate the exchange among centers of compatible data relating to cancer as a disease. The system should permit the development of collaborative studies into etiology and treatment response. Expected benefits include decreased time between initiation of a research study and its completion, the capability to study rare cancers, and the capacity for more definitive clinical and etiologic research than has previously been possible. The system now being organized will provide the basis for planning such studies and an organized structure within which specific research proposals can be planned and executed.

Between 1975 and 1977, 22 of 25 major institutional affiliates of 19 comprehensive cancer centers have organized at least minimal capabilities for the improved organization and management of research data; the minimum data set has been defined; a coordinating center has been established; and pilot operation of the system is scheduled for 1977-1978.

To improve communications between cancer centers and between the cancer centers and NCI, a center directors' meeting was held in October 1976. This was an occasion when center directors, Center Program staff, and representatives from other NCI divisions would discuss problems of mutual concern and items of mutual interest. A similar meeting is planned for November 1977.

CENTERS PROGRAM

(Dollars in Thousands)

	NONCOMPETING NO.	NONCOMPETING AMOUNT	COMPETING NO.	COMPETING AMOUNT	TOTAL NO.	TOTAL AMOUNT
CANCER CENTER SUPPORT (CORE)	52	$37,451	12	$19,249	64	$56,700
EXPLORATORY PROJECTS	6	673	7	527	13	1,200
CANCER CENTERS PATIENT DATA SYSTEMS	0	0	16	1,431	16	1,431
TOTAL	58	$38,124	35	$21,207	93	$59,331

RESEARCH FACILITIES CONSTRUCTION BRANCH PROGRAM

Description

The Cancer Research Facilities Construction Program was initiated in late September 1971 with a public announcement of the availability of limited grant funds to create additional cancer research facilities. The essence of this announcement is set forth in the following paragraph:

> In accordance with the President's call for an expanded, intensified, and coordinated cancer research program, and under authority provided by the Congress in the fiscal year 1972 appropriation act, the National Cancer Institute initiated a program of grant-supported construction of cancer research facilities. The intent of this program is to create new physical resources for cancer research through Federal participation in the cost of new construction and renovation. Support may be provided for the construction of facilities such as basic research laboratories; clinical research facilities; animal facilities; and basic associated core, administrative, laboratory, and service space. In all instances, the facilities proposed must be intended for expansion of cancer research for at least 20 years. A purpose of this program, in addition to strengthening research capabilities at existing cancer centers, is to develop new strong multidisciplinary cancer efforts in regions of the country where they do not exist. Thus, in the consideration of applications some attention will be given to geographical distribution as well as to the relation of proposed construction to existing centers of excellent cancer research.

Justification of construction funds has been, and will continue to be, in terms of new cancer research programs and safe facilities for accomplishing biohazardous cancer research. The primary review and evaluation of applications have been essentially scientific, considering such matters as scientific merit of the proposed program(s), the technical competence of the applicant institution staff, the intellectual environment of the applicant institution, and its scientific, fiscal, and administrative capabilities. In addition, other criteria have been considered, such as the location of the applicant institution, the applicant institution's role in the National Cancer Program, the promptness with which construction can be started, space requested commensurate with projected program scope, essential minimal facilities for those institutions with an undeveloped cancer research program, net space utilization of 60 percent or more, reasonable cost per gross square foot, acceptable design criteria, etc.

During fiscal year 1977, 11 construction grant applications were recommended for approval by the National Cancer Advisory Board. The requested NCI funding for these grants totals $23,800,000. In addition, three grants requesting

an additional $3,800,000 were carried forward from fiscal year 1976 as partial payments. Thus, the program faced a total obligation exceeding $27 million in fiscal year 1977.

The Construction Program's fiscal year 1977 budget was $16 million. These funds were awarded for the following projects:

- Completion of previous (fiscal year 1976) awards:
  Howard University
  Northwestern University
  University of Rochester

- Biohazard containment projects:
  Cold Spring Harbor
  Massachusetts Institute of Technology
  Ohio State University
  Roswell Park Memorial Institute
  Sidney Farber Cancer Institute

- Expansion of radiation therapy space:
  Yale University

- Construction of a new comprehensive cancer research center:
  Georgetown University

Recent Accomplishments

Policy changes were evaluated and recommended by the National Cancer Advisory Board. The following changes will be effective in fiscal year 1978:

(a) NCI funding will decrease from 75 percent of the eligible costs to 50 percent. This change will enable NCI to support more grant requests at a reduced funding level.

(b) The size of Construction grant requests which can be considered will be limited to $4 million per project. This change will accelerate the geographic distribution of cancer centers.

(c) Movable equipment may be included as an "eligible" cost on a formula basis for construction funding. Previously, these costs were not supported by construction grants and some facilities were only partially operational when completed. Therefore, NCI has decided to fund movable equipment during the construction phase to ensure that all completed projects are fully operational upon completion of construction.

CLINICAL CANCER EDUCATION PROGRAM

Description

Since 1948 the National Cancer Institute has encouraged improvements in the quality of education relative to cancer provided to physicians and dentists, the two health professions most concerned with the diagnosis and management of malignant diseases. Medical and dental schools were eligible to apply for grants to achieve a "coordination" of cancer teaching and to provide additional experiences for undergraduate students to expand their understanding of cancer in its basic and clinical aspects. In 1966, this grant program was revised to include stipends for graduate trainees in clinical oncology in appropriate specialties, and to promote educational activities relative to cancer for practicing physicians and dentists. In addition to medical and dental schools, schools of public health, cancer institutes, and major teaching hospitals affiliated with medical schools became eligible for these grants. In the interval between 1966 and 1975, grants were awarded to a total of 112 institutions, and more than 4,500 individuals received support for periods of training relative to cancer ranging from three months for undergraduate students to two to three years for graduate trainees who received instruction chiefly in medical oncology, surgical and gynecologic oncology, pediatric oncology, cancer pathology, and radiation therapy.

In 1974, following a general phase-out imposed on all training programs at the National Institutes of Health, a new program known as the Clinical Cancer Education Program was initiated to carry forward the objectives of the earlier programs, namely, to stimulate and expand multidisciplinary efforts in cancer education at the undergraduate, graduate, and continuing education levels so that physicians and dentists might deal most effectively with the clinical aspects of cancer. This new program, as did its predecessors, provides support by means of grants for selected faculty and staff, teaching equipment and supplies, consultants, and travel, but supports no trainees. However, undergraduate and graduate students may receive salaries for portions of time devoted to cancer educational experiences. A 24-member Clinical Cancer Education Committee reviews applications for these grants, visits applicant institutions, and advises on program needs and accomplishments. The major teaching activities supported by means of these grants include curricular review and analyses, development of core-curricula on cancer, cancer courses, establishment or expansion of multidisciplinary cancer teaching experiences such as conferences, seminars, clinical rotations, electives, the development of teaching materials and aids, exposure to visiting consultants and to specialized cancer facilities, and periods of intensified education relative to cancer of varying duration. All of the activities for which support is provided must be multidisciplinary and all must be evaluated for their efficacy.

The first Clinical Cancer Education grants were awarded in 1975 to 51 institutions for a total budget of $5 million. In 1976, these grants were continued and 31 new awards were made possible by a budget of $7.8 million. The budget for fiscal year 1977 ($8.9 million) will permit the award of 67 ongoing and 22 new or renewed grants.

Accomplishments

Unlike research projects, the outcome of cancer education programs cannot be judged by the contributions published by grantees in scientific journals. Improvements in the scope and quality of cancer instruction, in the extent of multidisciplinary cancer teaching activities, in the numbers of undergraduate and graduate students seeking specialized periods of cancer instruction, and in the numbers of practitioners responding to educational offerings are described by all grantees in their progress reports. Ultimately, these activities lead to improvements in the quality of care provided to cancer patients by students and practitioners who benefit from these experiences. Site visitors to applicant institutions have been impressed with the extent to which the goals of the earlier programs have been achieved.

To assist grantees in evaluating their own cancer education activities, a manual entitled "Evaluation in Cancer Education: Guidelines and a Systematic Approach," developed by a grantee at the University of Iowa in 1976, was distributed to all institutions with active Clinical Cancer Education grants. This manual provides assistance with developing finite objectives for cancer educational experiences, and with measuring their achievement in terms of knowledge, skills, and attitudes.

The Clinical Cancer Education Committee has reviewed 51 applications during three meetings in 1977, and has visited 27 institutions. In addition, consultative site visits have been made to five institutions and eight follow-up visits were made to review progress of ongoing programs. Progress reports received from 51 grantee institutions were reviewed by members of the Clinical Cancer Education Committee at their meeting on September 30-October 1, 1976; thirty-four reports were considered satisfactory, and letters indicating this were sent to the grantees; further information was requested from 17 grantees and was submitted upon receipt to the reviewers. Progress reports on 81 current grants will be reviewed by the Clinical Cancer Education Committee at their meeting in September 1977.

A successful workshop on "Training of Gastroenterologists in Cancer" was held on November 18-19, 1976, in Bethesda. This workshop, cosponsored with the National Institute of Arthritis, Metabolism, and Digestive Diseases, brought together 40 specialists in gastroenterology, internal medicine, surgery, radiology, radiation therapy, pathology, and cancer research who discussed the roles of the gastroenterologist relative to cancer and the content of programs which would adequately prepare him for these roles. A summary report of the workshop has been prepared and distributed to all participants. Plans for a follow-up meeting are under way. The success of this meeting has led to preparations for a workshop on "Graduate Education in Oral Oncology" which will bring together 36 oncologists, educators, and representatives of dental specialties on June 13-14, 1977, to discuss the role of dental specialists in the diagnosis and management of cancer of the head and neck, and the appropriate education for such roles. This workshop is cosponsored with the National Institute for Dental Research.

Emphases and Projections

In order to justify the resumption of training for specialized clinical oncologists, particularly in the areas of medical oncology, pediatric hematology-oncology, radiation therapy, gynecologic oncology, surgical oncology, and cancer epidemiology, a contract was awarded in December 1975, to Geomet, Inc., Gaithersburg, Maryland, to determine finite needs in four of these specialties for which such data were not readily available, on the basis of current and projected volumes of cancers of the types most appropriate to their specialized expertise. The results of this study indicate that there is currently a shortage of 1,991 medical oncologists, 285 pediatric hematology-oncologists, 666 gynecologic oncologists, and 2,373 surgical oncologists, based on agreed-upon average work weeks for each specialty. For 1980 and 1985 the shortages will increase unless current supplies of such specialists are expanded. These data will be used to justify the establishment of a Specialized Clinical Cancer Training Program which would provide stipends for graduate trainees in the clinical oncologic specialties in a manner similar to the ongoing institutional research training grants.

Additional projects which are planned include the development of criteria for cancer education in medical and dental graduate training programs and in the training of other health professionals, such as nurses, physician assistants, and dental hygienists.

Other Program Activities

Coordination is maintained with the Training Branch, National Cancer Institute, the Resources Branch, Division of Cancer Control and Rehabilitation, NCI, and to a lesser extent with the Office of Cancer Communications, NCI. Active participation is maintained in the activities of the American Association for Cancer Education and the American Cancer Society. Consultants to the Program include, in addition to the members of the Clinical Cancer Education Committee, numerous clinical oncologists throughout the country who are experts in cancer education; 50 such individuals have provided services to this Program in the past year.

CLINICAL EDUCATION

(Dollars in Thousands)

NONCOMPETING		COMPETING		TOTAL	
NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT
67	$7,013	17	$1,983	84	$8,996

RESEARCH MANPOWER BRANCH

Description

The Research Manpower Branch supports the training of scientists who will be the mainstay of the cancer research effort ten or fifteen years hence. Because no one can foresee the direction cancer research will be taking then, it is imperative that a broadly trained nucleus of investigators be maintained who can change their research interests as new leads develop and old specialties become obsolete. To that end, NCI supports research training in the scientific specialties composing the broad areas of cancer etiology and prevention, cancer detection and diagnosis, and cancer treatment and restorative care. The training may be in clinical or nonclinical specialties and may be in either basic or applied research. Three grant mechanisms are used to support research training, namely:

a) the Institutional Fellowship Award;
b) the Individual Fellowship Award; and
c) the Research Career Development Award.

The first two of these awards were created in 1975 by the National Research Service Act. Institutional awards are made competitively to qualified institutions to develop or enhance research training opportunities at the predoctoral or postdoctoral level. The applicant must have the staff and facilities for the proposed program. After the award is made, the institution's training program director is responsible for selecting the trainees and for administering the program. Residencies may not be supported by this program.

Individual fellowships are awarded only for postdoctoral research training in nonprofit institutions here and abroad. An applicant for a postdoctoral fellowship must establish his acceptance by a preceptor and must present a detailed description of the research project which he will undertake as part of his research training. Residencies may not be supported by this program.

Research career development awards are not governed by the National Research Service Act. The purpose of these awards is to provide very promising young investigators the opportunity to devote full time to their development as competent independent cancer investigators. Applicants must demonstrate appropriate scientific experience and achievement and must have outstanding research potential.

Accomplishments

During the year, 1,208 trainees and fellows and 110 research career development awardees were supported. Their distribution in the three broad research training areas was as follows:

Institutional Fellowship Awards
(training grants)

	Predoctoral	Postdoctoral
Cancer Etiology and Prevention	296	308
Cancer Detection and Diagnosis	18	42
Cancer Treatment and Restorative Care	56	113

Individual Postdoctoral Fellowships

Cancer Etiology and Prevention	262
Cancer Detection and Diagnosis	22
Cancer Treatment and Restorative Care	91

Research Career Development Awardees

Cancer Etiology and Prevention	69
Cancer Detection and Diagnosis	11
Cancer Treatment and Restorative Care	30

One hundred fifty-six postdoctoral fellows completed their training this year, as did 20 predoctoral and 52 postdoctoral trainees.

Some of the notable research accomplishments in the past year by postdoctoral fellows, trainees, and research career development awardees include the following:

The major flame-retardant compound used in children's pajamas, tris-(2,3-dibromopropyl) phosphate was found to be mutagenic. When absorbed through the skin or ingested from clothing, tris-BP poses a serious hazard as a potential carcinogen and mutagen (Blum et al., 1977).

By using three in vitro systems to study the mechanism of synthesis of DNA in human cells, a postdoctoral fellow has shown that RNA serves a primer function for the synthesis of nascent DNA. This in turn, is the precursor to high molecular weight DNA in lymphocytes (Tseng, et al., 1976).

The activation of endogenous leukemia viruses in mouse cells was achieved in actively dividing cells by exposing the cells to low dose-rates of gamma radiation. The requirement of cell division for radiation activation may reflect a mechanism for the initiation of virus expression in common with chemical activation (Otten, et al., 1976).

Studies of a new class of biologically active macromolecules, 5-thiolated polynucleotides, showed that some are potent selective inhibitors of DNA polymerases from various tumor viruses and from cultured Burkitt lymphoma cells. These chemicals act as inhibitors by competing with the structurally

analogous functional templates for sites on enzymes (Pafenholz, et al., 1976).

Nineteen acute leukemia patients in continuous complete remission for at least one year received late intensification therapy, after which they received no further chemotherapy. Most received BCG immunotherapy. Fourteen patients were still in complete remission two years later. The results support this therapeutic approach for long-term control of leukemia in adults (Bodey, et al., 1976).

Two anthracycline antitumor antibiotics, adriamycin and daunomycin, have been tested for tumorigenic activity. The results confirm previous findings that they can induce mammary tumors in female rats receiving single intravenous doses (Marquardt, et al., 1976).

One hundred twenty-four patients with hematologic and solid neoplasms were studied who had pretreatment urine polyamine determinations. Polyamine levels were found to be directly related to disease activity and tumor burden (Durie, et al., 1977).

A simple method has been developed for directly determining the superhelix density of closed circular DNA's by ethidium bromide titration using agarose gel electrophoresis in tubes containing increasing concentrations of the dye (Espejo, et al., 1976).

An analysis was made of the mechanism by which Friend virus (FV) suppresses T- and B-cell mitogenesis *in vitro*. FV mediates its suppressive effect on T- and B-cell mitogenesis through a suppressor cell which requires thymic influence for maturation, bears Thy-1 antigen, and is sensitive to lysis by cortisol (Kumar, et al., 1976).

A hamster fetal cell clone has been developed which serves as both an *in vivo* fibrosarcoma model and a test substrate for *in vitro* chemotherapeutic studies. Highly reproducible quantitative *in vitro* chemotherapeutic data can be obtained with this cell line within five days (Benedict, et al., 1976).

Emphases and Projections

A reliable and accurate estimate of future research manpower needs may be impossible to achieve, given the variables of long-term changes in the magnitude of research support, the constantly changing direction of future research emphases, and the technological limitations inherent in research manpower estimates. A number of cancer research manpower need estimates have been made during the last five years, including those done by various professional societies, the National Cancer Institute Cancer Research Training Committee, etc. In short, the sum of these estimates *in toto* is to identify as shortage areas virtually all the scientific specialties relating to cancer, i.e., radiology, oncology, medical physics, pharmacology, toxicology, epidemiology, pathology, cancer biology, carcinogenesis, immunology, etc. These estimates appear to be highly subjective in methodology. In 1976, the planning for a more objective manpower-needs study to be done under contract was completed.

Final clearance of the project by NIH is awaited. In the meantime, we have adhered to the Cancer Research Training Committee's advice in its 1971 report that "The eventual solution of the cancer problem will require the sustained labors of the broadest possible collection of experts in every area of biomedical science."

A conference of training grant program directors is being planned for fiscal year 1978. It is imperative that these people consider why only six percent of the National Cancer Institute postdoctoral research fellows and trainees hold the M.D. degree, and recommend steps to increase the number of post-M.D. trainees in NCI's Research Manpower Program.

RESEARCH MANPOWER PROGRAM

(Dollars in Thousands)

	NONCOMPETING NO.	NONCOMPETING AMOUNT	COMPETING NO.	COMPETING AMOUNT	TOTAL NO.	TOTAL AMOUNT
INSTITUTIONAL FELLOWSHIPS	98	$13,153	5	$514	103	$13,667
INDIVIDUAL FELLOWSHIPS	107	1,139	179	3,420	286	4,559
RESEARCH CAREER DEVELOPMENT AWARDS	99	3,118	6	191	105	3,309
RESEARCH CAREER AWARDS	8	240	0	0	8	240
TOTAL	312	$17,650	190	$4,125	502	$21,775

REFERENCES

Benedict, W. F. and Khwaja, T. A., A New Hamster Fibrosarcoma Model for In Vitro/In Vivo Evaluation of Cancer Chemotherapeutic Agents. Med. Ped. Onc. 2:271-278, 1976. (CA 70996)

Blum, A. and Ames, B., Flame-Retardant Additives as Possible Cancer Hazards. Science 17-23, January 7, 1977. (CA 05731)

Bodey, G. P., Freireich, E. J., Gehan, E., McCredie, K. B., Rodriquez, V., Gutterman, J., and Burgess, M. A., Late Intensification Therapy for Acute Leukemia in Remission. JAMA 235:1021-1025, 1976. (CA 00127)

Durie, B. G. M., Salmon, S. E., and Russell, D. H., Polyamines as Markers of Response and Disease Activity in Cancer Chemotherapy. Cancer Res. 37:214-221, 1977. (CA 00072)

Espejo, R. T. and Lebowitz, J., A Simple Electrophoretic Method for Determination of Superhelix Density of Closed Circular DNA's and for Observation of Their Superhelix Density Heterogeneity. Analytical Biochem. 72:95-103, 1976. (CA 00141)

Kumar, V., Caruso, T., and Bennett, M., Mechanisms of Genetic Resistance to Friend Virus Leukemia. J. Exp. Med. 143:728-740, 1976. (CA 70879)

Marquardt, A., Philips, F. S., and Sternberg, S. S., Tumorigenicity In Vivo and Induction of Malignant Transformation and Mutagenesis in Cell Cultures by Adriamycin and Daunomycin. Cancer Res. 36:2065-2069, 1976. (CA 00127)

Otten, J. A., Quarles, J. M., and Tennant, R. W., Cell Division Requirement for Activation of Murine Leukemia Virus in Cell Culture by Irradiation. Virology 70:80-87, 1976. (CA 00157)

Pafenholz, V., Hung, V. L., Yau-Kwan, H., and Bardos, T. J., Uptake of Partially Thiolated DNA by Ascites Tumor Cells. Cancer Res. 36: 1445-1452, 1976. (CA 02065)

Tseng, Y. and Gordian. M., DNA Synthesis in Human Lymphocytes: Intermediates in DNA Synthesis. J. Mol. Biol. 99:317-337, 1975. (CA 51862)

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